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PHYSIOLOGICAL REVIEWS

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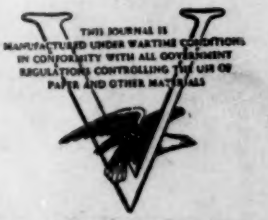
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HISTORY OF THE AMERICAN PHYSIOLOGICAL SOCIETY

To commemorate the Semicentennial of its foundation the American Physiological Society has published an illustrated history, with index, of its development since 1887. The book contains articles by William H. Howell and Charles W. Greene, with a foreword by Walter J. Meek, and was distributed in May, 1938.

The book of about 220 pages is in case-binding and costs $2.50.

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PHYSIOLOGICAL REVIEWS

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THE BENIGN MELITURIAS

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The term melituria usually designates the appearance of any sugar in the urine in amounts which can be detected by the reagents commonly used for the purpose. Small amounts of reducing substances are always found in minute quantities, but their presence can only be detected by the use of special methods.

It is questionable whether these substances are hexoses. Neuwirth (46) and others have observed that some of the material is fermentable in part. Peters and Van Slyke (49) came to the conclusion that normal urine does not contain more than 0.002 per cent of glucose and that the rest, reactive to alkaline copper solutions, consists of substances other than glucose, presumably glucuronides, ascorbic acid, phenols and unknown carbohydrates of possible ketone nature.

The quantitative appearance of any substance in the urine depends on its renal threshold. Due to the fact that this paper deals, to a considerable extent, with the glycosurias, this renal function is briefly discussed. The threshold for glucose is commonly given as 150 mgm. to 180 mgm. In a series of blood sugar studies by this author and co-workers (6, 19, 7) it was found that the accepted maximum can be considerably exceeded (220 mgm.) without glycosuria becoming evident. Benedict and Osterberg (4) consider the glucose threshold "wholly an artifact." They admit that the causes leading to hyperglycemia and subsequent glycosuria are usually the same, but question the necessity of a causal relationship of the two phenomena. In an earlier paper the same authors (3) claim that "the normal organism is truly diabetic in that it has no absolute tolerance for carbohydrate." For further information, the reader is referred to the very thorough discussion of this question by Macleod (35) and to the work of Campbell and collaborators (9).

The proposal of Lusk (34) that we must distinguish between glycosurias caused by an overstrained *sugar holding capacity* of the organism on one hand and the glycosuria of diabetes mellitus, due to an impaired *sugar burning* function on the other, will be of help in the classification of the various meliturias.

Alimentary glycosuria becomes evident whenever the sugar holding capacity of the body is exceeded. This capacity is influenced by the dietary habits of the individual, i.e., it depends on the question whether the habitual intake of carbohydrates is large or small. It is a well-known fact that the ingestion of a given amount of glucose, followed by an equal dose after a certain amount of time, produces first a rise in the blood sugar which is followed within an interval of one hour or so by a fall. This is known as the Staub-Traugott (58, 60) effect, although it was observed earlier by Hamman and Hirschman (22). In attempt-

ing to diagnose an alimentary glycosuria resulting from a supposed overloading, we must ascertain if the excessive intake took place on a fasting or nearly empty stomach. It is known that alimentary glycosuria is more apt to occur under this condition as observed by Goldblatt and Ellis (20).

According to Joslin (25), the following precautions should be taken whenever alimentary glycosuria is encountered: 1, correct diagnosis, 2, medical supervision, and 3, blood and urine tests at least yearly.

Renal glycosuria, erroneously referred to as renal diabetes or diabetes innocens, is a condition in which urinary glucose is found throughout the day even in the overnight fasting specimen. It is associated with normal carbohydrate utilization as expressed by the R.Q. The amount of sugar is largely independent of the diet and the threshold is low (56–100 mgm.). The condition is in all probability due to an impairment of the tubule cells, affecting the function of reabsorption of the sugar. Phlorhizin produces renal glycosuria. It also inhibits markedly the phosphorylation processes of the body. Because of these facts, the conclusion presents itself that the diminished resorptive power of the kidney in renal glycosuria is due to a disturbance of the phosphate exchange. The condition is rare. Joslin and co-workers (25) report the incidence of 62 cases in 18,000 meliturias, 9 of the above being renal glycosurias of pregnancy. Fowler (16) found 7 among 4000 and Marble (39) records 16 renal glycosurics in 9000 patients with urinary sugar.

It is extremely doubtful that renal glycosuria may progress to diabetes mellitus. Joslin (25) had 45 cases under observation for a period of 10 years or more. Not one of these developed diabetes mellitus. In one of the cases reported, sugar in the urine was discovered at the age of ten, in quantities rising occasionally to 5 per cent or more. The person was 58 years old in 1940, is on a slightly restricted diet, in good health, the father of five normal children.

The *renal glycosuria* of pregnancy was observed by Frank and Nothmann (17) and by Nürnberger (48). Pillman-Williams and Wills (50), as well as Rowe, Gallivan and Matthews (52), report the appearance of urinary glucose of benign aspect during the antepartum period in 35 to 60 per cent of the cases studied. The blood sugar levels were nearly normal, with a tendency to fall as pregnancy progressed. Only its impermanence distinguishes this type from ordinary chronic renal glycosuria. The condition has been used as the basis of a pregnancy test by Welz and Van Nest (62).

When renal glycosuria is suspected, carbohydrate tolerance tests and identification of sugar are of prime importance. Dietary rules should include a restricted intake of actual sugars, with directions for a liberal caloric sustenance. The patient should remain under his physician's care until the harmless character of the condition is firmly established. The normoglycemic glycosuria of pregnancy should receive special attention because cases have been reported where diabetes mellitus developed in the second pregnancy.

Emotional (psychic) glycosuria. As early as 1878 it was observed that excitement or pain caused the appearance of sugar in the urine. Böhm and Hoffmann (8) found that when cats not under anesthesia were tied to the operating table,

and when tracheotomy was performed, glycosuria of several hours' duration was observed. They called this phenomenon "Fesselungs Diabetes." Cannon and his co-workers (10) found urinary sugar in cats following fright or rage. However, under the same experimental condition, no glycosuria was noticed after adrenalectomy. This observation conjoined with the fact that normal animals showed an increase in epinephrine secretion under excitement pointed to the influence of the adrenals on the blood sugar level and carbohydrate metabolism. Cannon first used the term "emotional glycosuria".

Folin and associates (15), as well as Malmirwirta and Mikkonen (36) found glycosuria in students after course or subject examinations, the percentage of glycosuria in the group increasing with the severity and importance of the tests. Psychic or emotional glycosuria was observed in soldiers under fire, as cited by Mieth (43) and by Marañon (38) in aviators. Cannon (11) found urinary glucose not only in football players actively participating in the game, but also among the substitutes on the bench, as well as in spectators. Mann (37) observed transient glycosuria when patients were informed that they would have to undergo surgery. Kooy (27) explains the glycosuria of melancholia and manic excitement by the fact that the emotional state is responsible for the high blood sugar, the melancholic depression differing from normal grief by being mixed with anxiety, and manic excitement varying from normal joy because of its association with irritability.

A doctor's thesis by Mieth (43) is of decided interest in the present problem. It deals in detail with the psychic influences on carbohydrate metabolism not only from the position of sex, family or race, but also from the standpoint of occupational factors.

Emotional glycosuria of transitory nature can certainly be called harmless. If, however, the underlying cause is a state of prolonged worry and anxiety then the problem must be considered from the possible "diabetogenic" angle. Von Noorden (47) points to the fact that "high strung" people are more apt to become diabetics than the phlegmatic type. Lichtwitz (33) classifies certain vocations as "diabetogenic professions", i.e., callings where the productive attempt is coupled with psychic excitement and heavy responsibilities. The wealth of observations relative to the psychic or emotional influence on the metabolism of sugars should serve to remind us that the mental state of the diabetic must receive serious attention relative to the proper management of the disease.

Lactosuria. An increase in the reducing power of the urine of a pregnant woman was first observed by Lehman in 1850 (31). Hofmeister (23) obtained from the urine of a parturient woman a crystalline substance which had all the properties of lactose. Watkins (61) and later observers find that lactosuria occurs in varying intensity toward the end of pregnancy. A sudden marked increase of lactose is noted during the last two or three days before delivery, the maximum level being reached at the time of parturition. After delivery there is an immediate drop and this low level is maintained from two to six days. This is then very frequently followed by an abrupt and often tremendous increase in the sugar elimination, with subsequent marked fluctuations, lasting for about one month.

Thereafter, the urine lactose assumes a lower and more constant level. The subsequent downward trend is usually augmented by the weaning process. It might be of interest to mention that the tolerance to lactose is increased during menstruation. In the intramenstrual period it is the same as that of normal men, the tolerance dose being about 10 grams.

Galactosuria has been observed in sucklings and in patients following a galactose liver function test. In infants as well as in adults, the condition has been known to occur but always under pathological conditions usually involving the liver or the spleen. Such observations are found in the publications of Göppert (21), Bansi (2) and Mason and Turner (42).

Essential fructosuria (*levulosuria*) is a rare abnormality, a benign congenital error of carbohydrate metabolism. In normal individuals 80 per cent of the ingested fructose is converted to glycogen, the remainder going to lactic acid. In a case of fructosuria, 10 to 20 per cent of the ingested levulose is excreted instead of being metabolized. According to Sachs and collaborators (53) and to Silver and Reiner (56) the disturbed fructose metabolism is possibly due to the lack of a specific enzymatic action of unknown location, probably in the liver, intestine or blood. Jacobson (24) ascribes the phenomenon to an increased permeability of the kidney to this particular sugar. Fructose is less rapidly absorbed from the intestine and is utilized at a rate of only one-tenth of that of glucose. Soisalo (57) determined the fructose tolerance of three normals giving 1 gram of the sugar per kilogram of body weight. He found the highest level at 5 to 6 mgm. per cent and the renal threshold just below this figure. Joslin (25) gives a threshold value of 11 mgm. per cent (1 case).

Fructosuria was first observed by Zimmer (63) and at the present date there are approximately 60 cases reported in the literature. It is generally assumed that there is only one fructosuric in more than 100,000 individuals. Joslin (25) reports three cases in a study of 18,000 meliturias. A thorough review of the most typical cases can be found in the work of Sachs and co-workers (53).

The condition persists throughout life, the fructose being found in the urine in a fixed relationship to the intake. If a suspected fructosuric is given glucose there will be no, or very little, increase in the reducing power of the urine whereas if the fructose intake is augmented, the rise in urinary sugar can be noticed within a short time by semi-quantitative or quantitative tests (Benedict's). This simple procedure may be of help where only the simplest laboratory facilities are available.

The probable existence of a hereditary factor has been mentioned by some observers. Lasker (30), after a careful study of this problem, comes to the conclusion that "three classes of evidence indicate that fructosuria is inherited as a Mendelian recessive: fructosuria is common among brothers and sisters; it is absent in the parents and children with fructosuria; and parents are frequently consanguineous."

To establish a positive diagnosis we must have levo-rotation on polariscopic examination, absence of reduction of the usual sugar reagents and no optical activity after fermentation, a normal dextrose tolerance curve and identification

of the fructose by special tests. The Selivanoff test (55) is the most widely known. While this procedure is reliable in the hands of the experienced technician, it must be remembered that the typical red color can also be obtained with glucose if the heating is too prolonged. A test that can be performed with the simplest equipment is described by Lasker and Enklewitz (28). The procedure is as follows: 1 ml. of urine is added to 5 ml. of qualitative Benedict's reagent in a test tube. The mixture is heated in a water bath for ten minutes at 55°C. A yellow precipitate forms if fructose is present. Glucose does not reduce under these conditions. A white precipitate may be due to phosphates. Pentoses (xylulose) will also react, but can be ruled out, if necessary, by special tests. Furthermore, the possibility that fructosuria and pentosuria could be present in the same individual is exceedingly remote. Quantitative tests have shown as high as three and one-half per cent of fructose, although the amounts commonly found are much less.

Fructosuria has supposedly been observed in rare cases of severe diabetes mellitus; one such case is described by Marble and Smith (40).

Pentosuria or xylulosuria is connected with the appearance of a five carbon sugar in the urine. The pentoses excreted are not the same as those found in various chemical combinations in the body tissues, such as ribose and desoxyribose, but in most cases are xylulose, formerly called l-xyloketose. The condition is of no clinical significance since the metabolism of carbohydrates is not impaired.

The mechanism that produces the most commonly occurring urinary pentose, i.e., xylulose, is not definitely known. Joslin (25) states that the output is increased following a diet high in plums, cherries, prunes and grapes. Enklewitz and Lasker (14) fed a pentosuric 5 grams of xylulose isolated from the subject's urine. The output of the sugar was increased by only 0.5 gram. A normal control showed no reducing sugar after the same dose. The authors conclude that both the pentosuric and the normal can metabolize xylulose when administered orally.

It may be that the mother substance will be found through a study of the glucuronic acid metabolism. Certain glucuronogenic drugs such as amidopyrin, codein, menthol and phenobarbital increase the sugar output whenever pentosuria prevails. These same substances also further the formation of glucuronic acid. If glucuronic acid itself is fed to a pentosuric, we find an increase in the output of the sugar. There is a close chemical relationship between glucuronic acid and xylulose, the latter is derived from the former by the splitting of one molecule of carbon dioxide.

The first case of pentosuria was described by Salkowski and Jastrowitz (54) who found the sugar in the urine of a morphine addict. Neuberg (45) isolated and identified a racemic arabinose in a urine. Some of the older investigators have reported arabinose and other pentoses, but with the advent of more modern methods, we must agree with Levene and LaForge (32) that the sugar occurring in pentosuria is most always xylulose and very rarely racemic arabinose.

All reported cases of pentosuria with one possible exception (44) have been

Jews or people of distinct Jewish lineage, with the males predominating. The earliest "onset" is reported by Garrod (18) in a child of eighteen months. Pentosuria is found in all ages and persists throughout life. Margolis (41) finds an incidence of eleven cases in 22,000 persons examined. Blatherwick (5) found about one in 50,000. Joslin (25) discusses nine cases, all of them Jewish, ranging from three years to thirty-two years in age, with seven males to two females. Five of these cases have been under observation for ten years or more, all with persistent melituria, but in good health and with no sign of progress toward diabetes mellitus. Only one of the above cases showed a blood sugar which was occasionally above normal, but practically no other symptoms indicative of true diabetes. There seems to be a preponderance of males among pentosurics. It must be remembered, however, that many of these are discovered among applicants for life insurance, where males outnumber the females. Among children under fifteen years of age, the literature reports seventeen boys and eight girls.

Pentosuria is of distinct familial tendency. Af Klercker (1) pointed to this possibility as early as 1912. Lasker and co-workers (29) after an exhaustive study of this problem, come to the conclusion that the conditions which permit or cause xylulosuria are inherited as a simple recessive anomaly. It is doubtful that there may be other contributory factors. Protas (51) suggests that nervous trauma or infection may be contributory, but there is a possibility that the factor of drug action was overlooked in the study of the problem.

There are indications that pentosuria may occur jointly with mild diabetes mellitus. Earlier workers report this observation, but at the same time admit difficulties in the positive differential identification of the sugars present. Moss (44) studied the case of a non-Jewish woman. Over a period of eleven days her glucose excretion varied from 0.00 gram to 3.04 grams, and the pentose from 0.7 gram to 2.4 grams.

The presence of both sugars complicates the management of the diabetes, because of hypoglycemic manifestations occurring with reducing sugar in the urine. Insulin has no influence on the pentose metabolism. Edelman and Reiner (13) describe two cases (Jewish males), one of them a mild diabetic, as indicated by glucose tolerance tests.

To arrive at a diagnosis of pentosuria we must have a positive identification of the sugar, an absence of clinical manifestations of diabetes mellitus, a normal glucose tolerance curve and, if possible, a history of pentosuria in the family. To differentiate between pentoses and other reducing sugar, the Bial test is commonly used. A green color is produced by pentoses. The color can be shaken out with amyl alcohol and a typical absorption band near the D line is observed on spectroscopic examination. A confirmatory test with aniline acetate paper is recommended. Glucuronates may simulate a positive pentose test with Bial's reagent. They can be removed by treating the urine with Merck's blood charcoal. As mentioned before, pentoses will react like fructose if the procedure of Lasker and Enklewitz (28) is followed. It must also be remembered that a urine containing xylulose will retain its reducing power almost indefinitely, whereas a glucose urine, unless very acid, will show a greatly diminished reduction or no

reaction to Benedict solution at all, if allowed to stand at room temperature or in an incubator overnight. The naphto-resorcinol reaction of Tollens (59) distinguishes between pentoses and glucuronides.

Fermentation methods may prove of distinct help in the identification of the sugars encountered in the various meliturias. It has been found that so-called baker's yeast, sometimes used for the purpose, is usually not pure enough. Other mycologic agents, such as the monilias and certain sugar-fermenting bacteria, can be obtained in pure culture and have been found very useful. For details, the reader is referred to the publications of Castellani and Taylor (12) and of Klein (26).

There are or may be a number of additional substances in the urine which give or simulate a positive Benedict reaction. Maltose has been reported in the urine of beer drinkers. No positive indentification of the sugar has been made. The phenomenon could be due to a deficiency or absence of the widely-distributed enzyme maltase. Alkaptone bodies will give a reduction with the alkaline copper reagents. The supernatant fluid, however, is brown or greenish brown instead of blue. Bismuth reagents are not reduced and there is no fermentation by yeast. Some derivatives of glucuronic acid will reduce Benedict's reagent. The glucuronides or benzoic or salicylic acid are hydrolyzed by alkali, the phenolic derivatives are not.

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EXTRINSIC FACTORS THAT INFLUENCE CARCINOGENESIS

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One of the most fundamental discoveries in the entire field of cancer research is the observation that certain chemical or physical agents are capable of inducing tumors when brought in contact with living tissue. This discovery was followed by extensive experimentation and by the accumulation of voluminous data on the production of neoplasms with the various carcinogenic agents. Detailed studies have been made of the relation of chemical structure or of specific wavelengths of radiant energy to carcinogenicity; of the elimination of chemical carcinogens from the body; of the variations observed among species, strains and tissues; of age, sex, heredity, hormonal and extrachromasomal influences; and of the extraneous physiological effects of the carcinogen. Most of this information has been summarized previously (34, 52, 54, 55, 75, 81, 87).

The purpose of the present review is to assemble the information available on various extrinsic factors that alter the action of the carcinogens. No attempt has been made to include experiments dealing with established neoplasms. In general, in studies of this type, animals are treated with a carcinogen and in addition some other factor is administered by any of several methods. The studies appear to be motivated not only by academic curiosity but also by the hope that sufficiently extensive information on factors that alter carcinogenesis might lead to an understanding of the carcinogenic process itself or, in other words, to the cause of cancer.

This objective is still far away, and from the larger point of view the available data, though extensive, are indeed quite fragmentary. One fundamental question of interpretation can be raised against each of the experiments discussed. To what extent is the observed result concerned with the carcinogenic reaction itself and to what extent is it merely an expression of a change in the metabolism of a specific carcinogen? To illustrate, the injection of benzpyrene dissolved in mouse fat results in fewer tumors than when it is injected in a vegetable oil (65). This might indicate the presence of an anticarcinogen in mouse fat, but it is equally possible that a more rapid elimination of solvent with a corresponding quickened removal of the carcinogen occurs when mouse fat is employed. Accordingly, the results cannot be properly evaluated until the means are available whereby the necessary distinction can be made.

Extrinsic factors may either accelerate or retard the formation of neoplasms, and they may be effective just prior to, during, or following the administration of the carcinogenic agent. Thus phrases such as, "the production of those biological changes which represent the latent period of carcinogenesis," "the preparation of the soil," "the precipitation of a tumor at a site previously rendered neoplastic" have come into use. For the purpose of the present discussion the terms proposed by Berenblum (29) will be followed:

Anticarcinogenic action: The inhibition of the process of carcinogenesis.
Cocarcinogenic action: The augmentation of carcinogenesis by a noncarcinogenic agent.

This occurs when the appropriate agent is applied concurrently with a carcinogen which is acting under suboptimal conditions.

Precarcinogenic action: The production of a preneoplastic condition. Such an effect would be demonstrable by a shortening of the latent period of carcinogenesis in subsequent treatment with a carcinogen, or by preparing the ground for the subsequent action of an epicarcinogenic agent.

Epicarcinogenic action: The production of tumors in a tissue previously rendered preneoplastic.

Metacarcinogenic action: The conversion of a benign into a malignant tumor.

As might be expected, the dose of carcinogen is extremely important in determining the rate of cancer production, and extensive information on this subject is available not only for the hydrocarbons (41, 42, 45, 55, 70, 75, 87, 113, 183), and for the azo dyes (5, 96, 132, 185), but also for ultraviolet irradiation (11, 35, 36, 37, 181). Unfortunately for a study of extraneous factors on carcinogenesis, doses frequently have been selected for the production of the maximum number of tumors in the shortest time, a technique obviously not suited for testing the influence of factors that might cause some alteration of carcinogenesis. Several investigators have shown that the effect of certain agents might be obscured when overwhelming doses of carcinogen were employed (13, 45, 65, 183, 197). A satisfactory procedure is to administer the carcinogen at a level that will induce tumors in about 50 per cent of the animals. If the agent has been previously shown to have an augmenting action, it is desirable to test its effect on carcinogenic doses which by themselves induce less than 50 per cent tumors; whereas if a known inhibitor is used, the dose of carcinogen can be increased somewhat.

THE INFLUENCE OF AGENTS ADMINISTERED DIRECTLY TO THE REGION OF CARCINOGENESIS. *A. Chemical Agents. Solvents.* One factor that can influence the carcinogenicity of a hydrocarbon is the medium in which it is dissolved. There appears to be good agreement that the development of neoplasms is retarded when crystalline hydrocarbons are injected subcutaneously by methods that leave a deposition of crystals within the tissues. Various investigators have shown that the injection of hydrocarbons as powders, pellets, or as crystals moistened with a lubricant produce tumors less rapidly and in fewer animals than when given in oil solution (6, 7, 162, 201). Furthermore, the incidence of tumors was lower and the latent period longer when hydrocarbon-cholesterol pellets were introduced into the subcutaneous tissues of mice (186, 187), but it is difficult to make direct comparisons with other methods because of the different amounts of hydrocarbon that come in contact with the tissues when cholesterol pellets are employed. Peacock and Beck (162) believe that the induction of sarcomas following the injection of hydrocarbons in various solvents is chiefly dependent upon the rate of absorption of the carcinogen.

Hydrocarbons have been applied to the surface of the skin in many solvents, but no marked differences have been observed among the common organic solvents (53) and benzene seems to have the greatest popularity, although dioxan (109), acetone (29) ether plus mineral oil (57), and benzyl alcohol (182) have the advantage of being less toxic than benzene. Twort and Twort (205) ob-

served a stimulation in the rate of carcinogenesis with dibenzanthracene, methylcholanthrene or benzpyrene when chloroform was used as the solvent. Crabtree (56) found that the average induction time for papillomas was significantly lower when ether plus 2 per cent of liquid paraffin replaced benzene as a solvent for benzpyrene or when acetone plus 2 per cent paraffin was used instead of benzene as the solvent for dibenzanthracene.

Fats and oils. Various fats and oils are among the most common substances employed as solvents for the carcinogenic hydrocarbons and a pronounced influence on tumor genesis has been observed with these substances. The incidence of tumors is high following the subcutaneous injection of carcinogens dissolved in vegetable oils (corn oil (13), cottonseed oil (179), sesame oil (190), arachis oil (10), olive oil (162)), synthetic glycerides (188), lard (6), or paraffin (69), whereas fewer neoplasms are produced when fatty extracts of animal tissues are used as the solvents (49, 65, 145, 151, 162, 212). Lard, an animal product, appears to be the single exception to this general rule.

Peacock and Beck (162) reported that the injection of 0.5 to 1 mgm. of benzpyrene dissolved in mouse fat produced only a few tumors whereas carcinogenic activity was pronounced when the solvent was olive oil, or a mixture of $\frac{2}{3}$ olive oil and $\frac{1}{3}$ paraffin. Morton and Mider (145) obtained only 1 tumor in 44 mice that had received subcutaneous injections of 0.25 mgm. of benzpyrene dissolved in a petroleum extract of mouse carcasses, while the same amount of hydrocarbon dissolved in sesame oil produced 36 tumors in 46 mice. Dickens and Weil-Malherbe (65) observed that the subcutaneous injection into mice of solutions of 0.3 mgm. of benzyprene in sesame oil or in arachis oil gave rise to tumors in a large proportion of the mice, while the same dose of benzpyrene in either the liquid or the solid fraction of mouse fat produced only a few tumors. For the suppression of carcinogenic activity, it did not appear necessary that the lipoid material be of homologous origin since a mixture of fats and lipids obtained from ox brain was equally effective (65).

On the contrary, Oberling and his collaborators (156, 157) and Shimkin and Andervont (188) did not observe any retarding effect of rat or mouse fat. The latter workers, however, used comparatively large amounts of carcinogen: 25 to 75 mgm. of benzpyrene (156, 157) or 0.5 to 1.0 mgm. of methylcholanthrene (188). More recent work by Bryan and Shimkin (42) and by Sall and Shear (183) indicates that about 0.02 mgm. of methylcholanthrene is sufficient for the development of tumors in 50 per cent of their mice.

Peacock and Beck (162) attribute the anticarcinogenic action of homologous fat to the more rapid elimination of the solvent from the injection site, with a corresponding rapid removal of the carcinogen. Certain observations of Dickens and Weil-Malherbe (65) while not necessarily contradicting this explanation, nevertheless fail to support it: *a*, "the persistence of subcutaneous lumps near the injection site of the liquid mouse fats, apparently indicating a very slow dispersion of the injected solution; *b*, the fact that the solid fraction from mouse fat, which might be expected to be even less readily dispersed, and which also gave rise to many persistent lumps was similarly effective in preventing tumor

formation." These investigators imply that certain animal fats contain substances that are anticarcinogenic.

Another explanation is that the differences observed in tumor incidence when the hydrocarbons are dissolved in various fats are due to varying amounts of ingredients that augment or retard the carcinogenic process itself. In a very extensive and careful study of the effect of solvents on carcinogenesis due to hydrocarbons, Leiter and Shear (113) found differences in tumor formation between two different fractions of good quality pure leaf lard. The lard was filtered at 38°C. for 24 to 36 hours through coarse paper. The fraction that was liquid at 38° and which passed through the paper was designated "lard filtrate," and the material remaining on the filter paper was designated "lard residue." When the lard filtrate was used as a solvent for benzyprene the incidence of tumors varied widely although tumors usually developed in a high proportion of the animals. The variations were attributed to different proportions of retarding and promoting substances in the various specimens of lard filtrate employed. Lard residue exercised a striking retardation of tumor genesis by benzpyrene. Fractionation of lard, roughly according to degree of saturation of the fatty acids, indicated that the more saturated samples caused the greatest inhibition of carcinogenesis. Furthermore, tristearin and tripalmitin also inhibited tumor formation (113).

Still another explanation for differences in tumor incidence with various fats was suggested by the observation that benzpyrene dissolved in mineral oil or in vegetable oil survived oxidation *in vitro* for many weeks, but that in the presence of linoleic acid, the hydrocarbon had almost entirely disappeared within three weeks (150). The addition of α-tocopherol to the linoleic acid greatly increased the time required for the disappearance of the hydrocarbon. Vegetable oils contain considerable amounts of antioxidants and their stability to autoxidation is well known (158), whereas animal fats contain only small amounts of inhibitols and are quite susceptible to rancidity (158). Thus a loss of benzpyrene might occur when it is dissolved in an ether extract of animal tissues. The injection of such a mixture would in effect, then, only result in the administration of less carcinogen. On the other hand, when a stable oil was used as a solvent, little destruction of the hydrocarbon occurred. The successful production of neoplasms with lard as a vehicle for the hydrocarbons suggests the possibility that lard is more stable to oxidation than are the ether extracts of animal tissue. It would seem wise to test this point.

Apparently the effect of fats on the genesis of tumors is a complex one and no single theory covers all the known facts. Furthermore, generalization is difficult because the influence of certain fats may vary according to the technique of administration. Thus, Watson (212) demonstrated that pinene tar mixed with an ether extract of rat tissues augmented the formation of skin tumors when applied to the surface but prevented tumor genesis when injected subcutaneously. Watson and Mellanby (213) painted the skin of mice with a petroleum ether extract of mouse fat before tarring and observed an enhanced formation of tumors. Morton and Mider (146) also noted that the "fat effect" varied with

the mode of administration. When a petroleum ether extract of mouse carcasses was used as a solvent for benzpyrene which was injected subcutaneously or applied directly to the skin, tumor formation was retarded, but when this same mouse fat extract was painted on the skin 20 to 30 minutes prior to the application of benzpyrene, formation of skin tumors was augmented. Baumann, Rusch and co-workers (15, 91) observed that tumors were formed faster when 0.3 per cent benzpyrene was applied to the skin in a solvent containing cholesterol in cottonseed oil than when benzene was used as the solvent. In a continuation of this problem, Lavik and Baumann (110) tested the effect of other fatty substances on the skin of mice that were also being painted with a solution of 0.2 per cent methylcholanthrene. The carcinogen was applied twice weekly for the first 2 months of the experiment, and either oleic acid, potassium oleate solution, cottonseed oil, or a 5 per cent solution of cholesterol in cottonseed oil was applied at the site of carcinogenesis on days when the hydrocarbon was not painted. A definite increase in tumor incidence resulted from the local applications. Twort and Twort (205) reported that oleic acid augmented the development of skin tumors and suggested that cells rendered abnormal by a few applications of benzpyrene quickly passed into the irreversible cancerous phase when stimulated by this fatty acid. Contrary to these findings, oleic acid has been shown to inhibit the activity of the chicken tumor virus (88). Tumor growth has also been inhibited by the injection of fatty acids (19, 152). In this connection, it would be interesting to test the effect of the more unsaturated linoleic acid on tumor formation.

Mineral oil, in contrast to most oils of biological origin, did not augment the genesis of skin tumors, and when this substance was used as a solvent for the surface application of methylcholanthrene it actually retarded carcinogenesis somewhat (176, 205). Recently Simpson and Cramer (189) noted that the carcinogenic activity of methylcholanthrene was almost completely suppressed when dissolved in anhydrous lanolin and applied to the skin, thus confirming an earlier recommendation of lanolin as a protective agent against tar dermatitis (204). Lanolin is a mixture of variable composition and is known to contain phospholipoids and sterols as well as neutral fats. Since cholesterol alone has little influence on carcinogenesis either of the skin (176) or of subcutaneous tissues (186), studies of the effect of phospholipids on tumor genesis appear to be indicated.

Certain oils applied to the ears of mice exposed to ultraviolet radiation caused a very considerable acceleration of tumor development (176): mineral oil, and cholesterol in cottonseed oil were particularly effective; cottonseed oil, olive oil, and wheat germ oil gave moderate acceleration; while linseed oil retarded tumor formation. The retarding effect of this latter oil was ascribed to the formation of a film of oxidized oil which decreased the penetration of the carcinogenic light. Because peroxide formation has been observed when cholesterol and certain oils are irradiated in air, two peroxides, benzoyl peroxide and hydrogen peroxide, were applied to the ears of mice receiving irradiation, but they were without effect on carcinogenesis. Glycerol was also ineffective. Furthermore,

none of several irradiated oils had any carcinogenic properties. The accelerating effect of the oils on carcinogenesis due to light was ascribed to the presence of a thin film that formed a smooth surface on the ears and thus decreased the reflection of light from the skin. Presumably under these conditions, more radiant energy penetrated to the deeper layers of the tissues. However, it is also possible that the activity of the cells themselves was altered by the presence of oil (176).

Virtually all known carcinogenic chemicals are soluble in fat, and the carcinogenic hydrocarbons are capable of inhibiting the autoxidation of fats *in vitro* (63, 64, 178). They might also interfere with fat oxidation within the cell. Furthermore, the fats, as a group, appear to be more effective in influencing the activity of a wider range of carcinogens than any other type of agent. It would appear, therefore, that no theory which attempts to explain the mechanism of carcinogenesis can cover all the facts unless some phase of fat metabolism is included within it.

Irritants. Another group of substances that affect the formation of tumors might be designated as "irritants" although not all irritating substances affect carcinogenesis. Irritation, itself is not an easily definable entity. "It is, in fact, a vague generalization representing many different kinds of injury or stimulation of the tissues. Attempts have been made to classify irritants according to their action on the epithelium, hair follicles, blood vessels, nerve endings, etc., and according to whether the effects on the epithelium are growth stimulating, astringent, desiccating, or emollient. Owing to the complexity of action in most cases, such attempts at classification have generally proved confusing and are often definitely misleading" (27).

Earlier writers have frequently considered irritation as a carcinogenic agent but recent evidence suggests that it is merely a contributing factor in carcinogenesis. Croton oil, a powerful irritant, happens to be one of the most potent cocarcinogens known. Berenblum (28) applied an acetone solution of 0.05 per cent benzpyrene to mice at weekly intervals and observed tumors in only 3 per cent of the animals. This incidence was increased to 37 per cent when 0.5 per cent croton oil was added to the solution of benzpyrene. Croton resin, (50) a constituent of croton oil, possessed cocarcinogenic action to an even greater degree; the incidence of tumors was increased to 80 per cent when the resin was applied with the benzpyrene. Both the croton oil and resin were demonstrated to be themselves devoid of even slight carcinogenic activity. The croton resin also precipitated tumor formation in areas previously treated with benzpyrene (29). A group of mice received 8 weekly applications of a 1 per cent solution of benzpyrene in acetone and the animals were then divided into three groups. In the first group, the skin was painted at weekly intervals with a 0.025 per cent solution of croton resin in acetone; in the second group, the skin was painted with 30 per cent of turpentine in acetone; while in the third, acetone alone was applied as a control. The painting was continued for 22 weeks, after which the animals were left untreated for a further 6 weeks when they were killed for histological examination. In the acetone control group 18 per cent had developed tumors

at the site of application, in the turpentine group 44 per cent of the mice had tumors, while those receiving the croton resin showed a tumor incidence of 86 per cent. Furthermore, fewer tumors regressed and more became malignant in the group receiving the croton resin than in the control group, while the turpentine series was intermediate.

Creosote oil is another substance that influences tumor formation, although, unlike croton oil, which acts only as a positive promoter of tumors, creosote oil has been separated into fractions three of which augment while four others retard neoplastic formation due to benzpyrene (45, 183). The retarding agents also caused damage to the skin and this might have been responsible for the retardation; however, a phenolic fraction which was anticarcinogenic produced no obvious injury to the epithelium. These fractions were tested by applying them to the mice simultaneously with the hydrocarbon; it would be of interest to determine their effects when administered after a preliminary treatment with a carcinogen.

As an example of an irritant that does not affect carcinogenesis, xylene has been applied to mouse skin in concentrations producing an irritation equivalent to that due to croton oil, without, however, altering the number of tumors that developed as a result of benzpyrene treatment (28). Although turpentine possesses weak epi- and metacarcinogenic activity (29), it, like xylene, was virtually devoid of cocarcinogenic activity for skin tumors caused by benzpyrene applied during the first phase of tumor formation (28). Furthermore, when benzpyrene was dissolved in a 30 per cent solution of turpentine in olive oil and injected subcutaneously into mice, abscesses were induced around the injection mixture, but this acute inflammatory reaction did not accelerate the genesis of tumors. On the contrary it appeared to diminish the incidence of neoplasms (16).

Still another group of chemicals that has a pronounced influence on carcinogenesis includes mustard gas and related compounds. Berenblum (24, 26) observed that the addition of 0.05 to 0.1 per cent of mustard gas (β,β' dicholorodiethyl sulphide) to a carcinogenic tar inhibited tumor production. Only 18 out of 240 mice developed tumors when a mixture of tar and mustard gas was applied to the skin, in contrast to an incidence of 131 out of 235 when tar alone was used under identical conditions. Of the compounds related to mustard gas, ethylene-bis-chloroethyl sulphide and dibromodiethyl sulphide, gave marked inhibition; dichlorodiethyl sulphone and dibromodiethyl sulphone gave moderate inhibition; while diiododiethyl sulphone, dichlorodiethyl sulphoxide, thiodiglycol and dichlorodiethyl ether were without effect (27). Among the irritants not belonging to the mustard gas group only cantharidin gave marked inhibition while cyclohexane elicited a slight retarding effect. Iodoacetic acid, trichloracetic acid, croton oil, acetic acid, "lysol", and turpentine were without influence.

Mustard gas is an oily liquid readily soluble in fats and fat solvents but only sparingly soluble in water. In the presence of water it is decomposed fairly rapidly into dihydroxydiethyl sulphide (thiodiglycol) and hydrochloric acid. It has been suggested that the irritant action of mustard gas is dependent on its high lipoid-water distribution coefficient and that the damage is due to the liberation of free HCl inside the cell (118). Furthermore, mustard gas has been shown

to react with amino acids (48) and this suggests the possibility of an interaction with the protein constituents of the cell. Berenblum, Kendal and Orr (30) attempted to determine the metabolic changes induced by mustard gas, etc., but no successful correlation regarding the rate of hydrolysis and the inhibition of metabolic processes could be made. Mustard gas lowered the glycolytic rate considerably while ethylene-bis-chloroethyl sulfide had little effect on this process.

Crabtree (57, 58) observed that certain concentrations of monochloracetone altered the rate of tumor formation due to benzpyrene, and this "chlor-compound" also inhibited glycolysis. Ninety mice were treated twice weekly with 0.3 per cent of benzpyrene in a solvent of 98 per cent of ether plus 2 per cent mineral oil. To one group of 30 mice 0.3 per cent monochloracetone was applied three times a week; 30 others received 3 per cent monochloracetone in the same way; while the remaining 30 were kept as controls. The low concentrations of monochloracetone caused a marked inhibition of tumor development but the higher levels were either without effect or they stimulated tumor development slightly. Monochloracetone is also capable, in a wide range of concentrations, of stimulating tumor induction in mice that have received a preliminary, but subeffective, treatment with 0.3 per cent benzpyrene applied for 8 weeks. Stearyl chloride, palmityl chloride, myristyl chloride, valeryl chloride, acetyl chloride and benzene sulfochloride when added to 0.1 per cent or 0.3 per cent benzpyrene retarded papilloma formation in mice (58). These latter "chlor-compounds" differ from monochloracetone in that they are hydrolyzable under physiological conditions. Since the one common feature of all these compounds is the active chlorine atom, Crabtree (58) suggested that the inhibition of glycolysis *in vitro* by mustard gas is also caused by the liberation of HCl by this compound.

Reimann and his associates (166) have demonstrated that the application of 0.5 per cent p-thiocresol to the skin of mice will protect it against carcinogenesis due to dibenzanthracene. The application of sulfhydryl compounds apparently speeds the rate of cell multiplication in the basal layer of the skin and with this stimulation, the cells proceed to higher degrees of differentiation and organization. Since neoplasms arise from incompletely differentiated cells, it is suggested that tumor formation will necessarily be decreased in tissues in which most of the cells are well on their way to complete differentiation. Potter (163, 164) has suggested that thiocresol protects the SH groups of certain enzymes that might otherwise be inhibited by carcinogens. Enzymes of both aerobic and anaerobic systems within the cells are known to contain SH groups. It has been suggested that agents that induce shifts from the aerobic to the glycolytic metabolism over a sustained period would, to this extent, duplicate one of the properties of carcinogens (163). Conversely an anticarcinogen might be expected to protect the SH enzymes against inactivation. Certain experimental facts were presented in support of this view. Metabolic split products of p-dimethylaminoazobenzene have been shown to inhibit enzymes concerned with glycolysis (93, 95) and with oxidation (163, 165) presumably by the inactivation of the SH groups. Although it is possible that a similar inactivation of SH enzymes could occur with the hydrocarbons, this has not been susceptible to testing thus

far because of the insolubility of the hydrocarbons in an aqueous medium. It is possible that these latter carcinogens make contact with enzymes which contain some lipoid in the molecule. Certainly more work in this direction is indicated.

Other compounds not classified as irritants have also been shown to influence the genesis of tumors. There is some preliminary evidence that xanthine, hypoxanthine, nucleic acid, histidine, nicotinic acid, d,l-phenylalanine, tyramine, tyrosine, and guanine decrease tumor incidence when added to solutions of filtered lard containing benzpyrene (133). The administration of small amounts of indole or arsenious acid to white mice for a period of 45 to 60 days prior to tar painting, has been reported to predispose the animals to neoplasms and to accelerate tumor development (51). Although weak carcinogenic properties have been attributed to arsenic (134) there is little unequivocal evidence to support this view (92). Since the question has arisen concerning the advisability of administering arsenic compounds to individuals under treatment for cancer, it appears that the effect of arsenic on experimental tumor genesis should be reinvestigated.

B. *Physical Agents. Injury, inflammation and healing.* Berenblum (25) has shown that the concurrent application of carbon dioxide snow and tar to the same area of skin inhibited the induction of tumors whereas an augmentation of tumor formation occurred if the treatment with carbon dioxide snow followed that with tar. Since carbon dioxide snow is itself a mild carcinogen (23), this result appeared to represent a summation effect.

Very similar observations were made by DesLigneris (62) who employed heat instead of cold to the skin of mice that also received applications of methylcholanthrene. A small cotton plug mounted on a forceps was plunged into boiling water, left in the air for about 2 seconds, and then applied to the skin by simple contact for about a second. This scalding alone produced no tumors, and when applied on alternate days with the methylcholanthrene, little effect was noted; if anything, the scalding diminished the number of tumors. However, when a three month period of hydrocarbon administration preceded the scalding, the latter treatment precipitated tumors in the preneoplastic area. In fact, the epicarcinogenic activity of the scalding was almost as great as that noted when methylcholanthrene had been continued in its place. Choldin (51) reported an acceleration of tumor genesis in mice if test tubes containing hot water were applied a short distance away from the area treated with tar, although no augmentation of carcinogenesis was noted when the thermal irritation was applied to the treated area. Parodi (161) found that the production of tar tumors was delayed if the skin had previously been heated to 70°C. for several months, while heating the epidermis to 50°C. favored the development of cancer. Lauridsen and Eggers (108) observed a slight acceleration in the development of neoplasms in the skin of mice if the area treated with dibenzanthracene also was cauterized at weekly intervals with a hot wire. The application of a caustic pencil or hot needle to the base of a papilloma stimulated malignant changes in mice whereas incisions of the skin made during the application of tar had no effect on tumor development (59). Brunschwig, Tschelter and Bissell (40) did not find an in-

crease in tumor formation when an area of mouse skin painted with benzpyrene was cauterized with a hot metal object.

Injury resulting from scarification or incisions has much the same effect as the trauma produced by thermal methods. Deelman and VanErp (61) and Deelman (60) stated that an incision made in the skin of a mouse in the neighborhood of a tar cancer led to the development of a new carcinoma, which appeared in the healing wound. These workers concluded that the skin close to a carcinoma is in a precancerous condition, so that the application of a trauma, insufficient to produce malignancy when applied to the skin elsewhere, will elicit the genesis of a neoplasm if applied to the skin in the neighborhood of a tar carcinoma. Orr (159) reported that fibrosis produced by placing threads in the subcutaneous tissues underlying the skin treated with dibenzanthracene led to a more rapid appearance of epithelial tumors. Ludford (117) used sandpaper to scarify the skin of mice both prior to and following treatment with tar, but observed only a slight retardation of subsequent tumor formation. The significance of this observation however, is open to question since many minute tumor nests could have been removed mechanically as rapidly as formed, a result which would be less apt to occur from simple incisions. In general it appears that neither thermal nor mechanical injury affects the development of skin tumors if such treatment is attempted too early in the carcinogenic process. However, trauma applied after the preneoplastic stage usually stimulates the formation of neoplasms.

Injury to the deeper tissues, however, appears to have no effect on the genesis of tumors arising from such areas. DesLigneris (62) was unable to accelerate tumor genesis by the injection of glass particles along with methylcholanthrene or benzpyrene. Similarly, Boyland and Burrows (38) observed no augmentation when fine silica was mixed with a colloidal aqueous suspension of dibenzanthracene. Woglom (220) injured glandular epithelium by drawing threads soaked in benzpyrene through various animal organs but this trauma plus hydrocarbon did not induce tumors in the various organs.

A rapid vigorous growth of liver tumor cells in healing wounds, which was soon followed by a regression of the neoplasm as the processes of repair neared completion, has been described (101). Three possible explanations for this effect were suggested: *a.* Tumor growth may be stimulated in an area of rich vascularization but retarded and finally stifled when the blood supply is decreased. *b.* The injured cells might elaborate one or more factors that stimulate growth. Substances of this category have been produced by injured yeast cells (116) and Menkin (129) has shown that an inflammatory exudate injected frequently into the subcutaneous tissue of a rabbit's ear will induce a severe inflammatory reaction. Cessation of these injections is followed months later by a sustained and marked proliferative activity. This is characterized by hyperplasia and metaplasia of the normal epithelial layer and by foci of keratinization. Furthermore, the cartilage of the ear, at the site of injections, manifested marked proliferative activity giving rise to small nodules. *c.* Injury to the cells may result in a decrease in local tissue resistance that permits the proliferation of cells otherwise held in check (59, 101).

Radiant energy. X-rays, ultraviolet radiation, visible light and infra-red rays all appear to exert some influence on carcinogenesis, and ultraviolet and x-ray are themselves carcinogenic.

Wallace, Wallace and Mills (210) reported that mice kept at 92°F. developed tumors due to methylcholanthrene more rapidly than did mice maintained in an environment of 65°F. Bain and Rusch (12) demonstrated that mice kept in a box maintained at 35°C. for 30 minutes while exposed to ultraviolet radiation developed tumors faster than controls irradiated at room temperature or at 3–5°C. Whether this effect was the result of the increased rate of tissue metabolism or to some other influence cannot be answered at the present time.

Visible light also has been reported to influence carcinogenesis with hydrocarbons. Doniach and Mottram (68) found that strong sunlight plus benzpyrene painting caused a dermatitis and reduced the incidence of tumors. This finding was verified by Morton and his collaborators (143, 144) who used artificial lamps for the source of light. Seelig and Cooper (184) were unable to demonstrate any influence of daylight on the effect of tumor genesis in mice painted with tar. Conflicting results have been reported by Vles, DeCoulon and Ugo (208, 209) and by Maisin and DeJongh (121) who noted an acceleration of tumor formation when mice painted with tar or benzpyrene were subjected to visible light.

The carcinogenic potency of x-rays can be augmented if abscess formations are also induced in the treated tissues. In a study of this type, Lacassagne (105) and Lacassagne and Vinzent (107) produced inflammation in the subcutaneous tissues of rabbits by the injection of *Streptococcus caviae* or by the injection of sterile diatomaceous earth. The lesions were submitted to x-radiation, the dose in each instance being 600 r. Such doses had a beneficial effect on the infective lesions but sarcomas eventually developed at the sites of irradiation in 5 of the 12 survivors that had the infected lesions and in 2 of the rabbits that had received the sterile diatomaceous earth. Burrows, Mayneord and Roberts (44) observed similar results. They induced a focus of inflammation in the groin of 12 rabbits by injections of kaolin and of finely powdered silica suspended in olive oil. These foci were exposed to 600 r. of x-ray at a single dose. Among nine rabbits thus treated and surviving for two years or longer, tumors appeared in the irradiated tissues of six. In four of these instances the tumors were sarcomata that produced metastases. X-ray alone in the same dose was non-carcinogenic.

Büngeler (43) has reported that the incidence of skin tumors in mice due to sunlight can be increased by the injection of certain photosensitizing agents such as eosin and hematoporphyrin. Others have suggested that oxidizing agents or substances that alter the oxidation-reduction potential might have some influence on tumor growth (176). However the following compounds failed to affect the production of tumors due to ultraviolet radiation: rose bengal, neutral red, dichlorophenol-indophenol, catechol, p-thiocresol, histamine, benzoyl peroxide, hydrogen peroxide, histamine, benzyl alcohol, and benzene (176, 182).

C. *Combination of Carcinogens.* The carcinogenic effects of the various hydrocarbons are apparently additive: that is, several different carcinogenic hydro-

carbons may be applied to the skin of mice in almost any order without interruption of the carcinogenic process (89, 111, 182). Apparently skin does not differentiate between the chemicals and responds as if one carcinogen were acting continuously.

Variable results are obtained, however, by combining carcinogenic chemicals with radiant energy. Mottram (147, 148) observed that the application of radium to the backs of mice previously treated with benzpyrene was followed by a greater number of epithelial tumors. This was true only if the dose of radiation was small. The tumors were found at a distance from the previous site of the radium applicator, in a region that had received from 800–2800 r. units. Taschner, Gottlieb and Spritzer (198) treated a series of mice with x-ray taking care to screen an area of the skin with lead. After one month a solution of methylcholanthrene was applied to that portion of skin which had been shielded from the x-rays. Tumor formation was accelerated in the mice that had previously received the larger doses (650 r.) while the lower dose (150 r.) had a slight retarding effect. Brunschwig, Tschelter and Bissell (40) were unable to confirm these results, probably because of differences in experimental procedure. The latter workers applied a 1 per cent solution of benzpyrene three times a week for only 2 weeks before the radium plaque was applied, whereas Mottram applied benzpyrene for 60 to 75 days before treating with radium. Furth and Boon (80) observed that leukemogenic action of small doses of methylcholanthrene is greatly enhanced by pre-irradiation with doses of x-rays.

When ultraviolet radiation was used in combination with tar, increased cancer formation has been reported (76, 200) but these observations have not been confirmed with pure hydrocarbons (176, 182, 199). The treatment of mice with ultraviolet radiation preceding, during or after periods of application of methylcholanthrene or of 9,10-dimethyl-1,2-benzanthracene did not increase the carcinogenic effectiveness of the chemicals, nor did the presence of the hydrocarbons augment the development of tumors due to the ultraviolet radiation (182). Actually there was a slight decrease in the number of neoplasms when both agents were used simultaneously. This inhibitory effect could have been due to a partial destruction of the hydrocarbon by ultraviolet light and to the absorption of radiant energy by the hydrocarbon. Benzpyrene is known to be unstable to light (149). In contrast to the hydrocarbons, tar is such a complex mixture that light might not readily affect its carcinogenic potency. Another explanation for the lack of augmentation between the two types of carcinogens is that they involve essentially different mechanisms that are incapable of summation under these conditions.

Observations have also been made on combinations of other dissimilar carcinogens. Rous and his collaborators (79, 119, 173, 174) noted that benign warts which had been induced in rabbit's ears by the application of tar became large and invasive after the intravenous injection of Shope papilloma virus, while neither agent by itself induced malignant tumors so quickly. Similarly, Ahlström and Andrewes (2) observed an intensified response to fibroma virus when tar was injected intramuscularly. However, Rusch and his associates (182)

were unable to demonstrate a summation effect by the combination of Shope papilloma virus and ultraviolet or x-irradiation, and Beck (17) did not observe an increase in the general susceptibility to cancer by cauterization, x-rays or ultraviolet light when mice were given intraperitoneal injections of benzpyrene.

THE INFLUENCE OF FACTORS ADMINISTERED AT A DISTANCE FROM THE REGION OF CARCINOGENESIS. A. *Diet. Caloric influence.* A fundamental observation that must be considered in all experiments involving the dietary modification of tumor development is the fact that tumors develop more slowly and in a smaller percentage of animals when the total intake of food is restricted in amount. Tannenbaum (195), for example, has shown that the incidence of spontaneous lung tumors in ABC strain mice was reduced by about 50 per cent in animals fed a restricted diet, and a striking reduction in the incidence of spontaneous mammary carcinoma in mice maintained on a calorie restricted diet has also been demonstrated (196, 207). In a group of 50 virgin female mice of the DBA strain fed *ad libitum*, 18 developed spontaneous breast tumors by the 86th week of life whereas no tumors formed in a similar group kept on a diet restricted as to calories (196). The results of Visscher and his co-workers (207) were even more marked: 67 per cent of a series of 51 female mice of the C_3H strain allowed unlimited food developed spontaneous mammary neoplasms by the 16th month as compared to no tumors in a similar group fed a diet which was limited in calories by 33 per cent. McCay and his associates (127, 128) observed a reduction in the number of spontaneous tumors in rats kept on restricted diets. Like spontaneous tumors, several types of tumors due to chemical carcinogens are also susceptible to the effect of a reduced caloric intake. Thus the incidence of skin epitheliomas induced in mice by applications of dibenzanthracene was approximately 90 per cent in the full fed control group and only 30 per cent in the experimental group receiving fewer calories; in mice that had received subcutaneous injections of benzpyrene, 82 per cent of the controls had sarcomas as compared to 47 per cent in the restricted group (196). There was also a delay in time of appearance of such neoplasms as developed. However, a reduced caloric intake had little or no influence on the subsequent growth of sarcomas induced by benzpyrene or of spontaneous breast tumors (196), although Bischoff, Long and Maxwell (32) have reported a retardation in the growth of sarcoma No. 180 in mice whose caloric intake was restricted by 50 per cent; less drastic reductions in food intake failed to yield significant results.

The opinion has been expressed that the effects of caloric restriction are observed only in poorly nourished, cachectic animals, and it may be true that in some of the earlier experiments caloric restriction was accompanied by a simultaneous reduction in the intake of protein, vitamins and salts since the diets employed were identical in composition but different in amount in the full-fed and restricted groups. However, in the more recent experiments cited above (196, 207) the restricted mice were fed amounts of protein, vitamins, and minerals, approximately equal to those ingested by mice eating the control diet *ad libitum*, while the carbohydrates and fats were reduced not only in total amount but also in percentage of the diet. Thus, the inhibition of tumor formation that

resulted could be attributed to caloric restriction *per se*. Some of these experiments were continued for over a year without any obvious nutritional deficiencies appearing in the animals. Few other experiments on nutrition have been continued for an equal span of time. Although the average weight of the calorie-restricted mice was 20 to 23 grams while the controls weighed 28 to 32 grams, the restricted mice appeared sleek and healthy and, in general, outlived the controls (196). McCay and his associates (128) also noted an increase in the life span of rats kept on restricted diets, but in his experiments, the growth of the rats was deliberately retarded to a gain of only 5 grams in 50 days.

The interesting suggestion has been made that the intake of calories might also influence the incidence of human cancer. Tannenbaum (194) has made a survey of insurance statistics and has suggested that cancer mortality increases with increasing body weight. Reasoning from experience with animals, it would appear that the avoidance of overweight might prevent or delay the inception of a tumor but would probably be of no value once the tumor has developed. That any conclusion at all should emerge from insurance statistics is the more significant when one recalls that the analyses of human statistics cannot have the accuracy of animal investigations in which pure strains of animals of known genetic composition, living in the same laboratory, are used, and where age, temperature, weight, diet and factors of carcinogenesis can be controlled. Furthermore, in an experimental investigation on animals, one may study a single type of tumor, in contrast to some statistical studies on man, in which all types of cancers are grouped together.

The effect of caloric reduction on carcinogenesis is so pronounced that this effect must be considered in interpreting any experiment involving the influence of secondary factors on tumor development. Thus, it is possible that the retardation of tumor formation by some agent may well be the indirect result of caloric restriction rather than the direct consequence of the therapy itself.

An indirect way of achieving caloric restriction in a mouse is to force it to perform physical work. When mice bearing transplantable neoplasms were subjected daily to periods of forced exercise, the rate of tumor growth was slower than that found in mice not treated in this manner (180). Conversely, the augmentation of tar cancer in rabbits following injections of glucose (172) might also be a caloric one.

Fat effect. There is abundant evidence that diets high in fat tend to increase the rate of formation of certain types of induced tumors. Watson and Mellanby (213) observed that the carcinogenic activity of tar was increased if the experimental mice were maintained on a diet to which 12.5 to 25 per cent butter had been added: 55 per cent of the mice fed the butter fat developed tumors as compared to only 28 per cent in the control group. Furthermore, 60 per cent of tumor-bearing animals in the butter fed group also had lung nodules compared to 37 per cent with adenomata in the control group (213). Vles, DeCoulon and Ugo (208) noted an augmentation of tar cancer when egg yolk was fed to mice. In a series of recent papers, Baumann and his associates studied the tumor-promoting effect of fat in some detail. Control mice receiving continuous applica-

tions of hydrocarbon solutions (methylcholanthrene, benzpyrene or dibenzanthracene) required approximately one month longer for 50 per cent of the animals to develop skin tumors than mice treated in the same manner but fed diets to which 15 or 20 per cent of fat had been added (13, 91). Dietary fat also markedly increased the number of tumors in mice treated with an amount of methylcholanthrene that was only sufficient for the production of tumors in a small percentage of the mice (109). In another experiment certain components of fat were investigated in an attempt to identify the active fraction. Mice fed 15 per cent of Primex (partially hydrogenated cottonseed oil) had a tumor incidence of 60 per cent; those given 15 per cent of ethyl laurate had a tumor incidence of 63 per cent, in contrast to 11 per cent in the control group. Glycerol and the unsaponifiable fraction of the fat were less effective. It appeared therefore that the fatty acid fraction was largely responsible for the fat-effect. The highest incidence of tumors appeared when fat was given throughout the experiment, but measurable increases were also observed when fat was fed either during the first two months while the carcinogen was applied or after the application of hydrocarbon had ceased. The most effective period was from 2 to 12 weeks after the beginning of the application of hydrocarbon (109). The fatty acids of hydrogenated vegetable oil, prepared free from unsaponifiable matter and resynthesized into triglycerides, had essentially the same tumor-promoting activity as natural fat (110). A definite, though lesser effect of fat on the incidence of skin tumors due to benzpyrene was observed in 3 different strains of mice by Tannenbaum (197). Furthermore, the incidence of spontaneous breast carcinoma was significantly increased in DBA virgin mice fed a high fat diet: 32 of 50 mice on the high fat diet eventually developed spontaneous breast tumors as compared to only 16 in a similar group fed a control ration. Baumann and Rusch (14) demonstrated that diets with a high fat content also accelerated the formation of tumors due to ultraviolet radiation.

At least two explanations of the fat-effect have been considered. The fat might act locally in the carcinogenic area, by altering the rate of hydrocarbon penetration of the skin or by accelerating the process in some other manner, or it might exert a more generalized systemic influence. The local effect is no doubt significant in the dietary experiments because mice fed high fat diets frequently exhibit a marked greasiness of their fur (176, 213). When mice were fed fat as an emulsion in the drinking water, this greasiness was diminished, and the fat consumed appeared to be only one-half as effective in increasing tumor formation as when the fat was mixed with the solid food (110). Lavik and Baumann (110) observed that mice painted with methylcholanthrene consumed 12 to 30 per cent more calories on diets high in fat than on corresponding low-fat diets, and a rough correspondence appeared to exist between the caloric intakes of the mice on the various rations and the numbers of tumors that ultimately developed. On an equivalent caloric intake, the incidence of tumors in mice on the high and low-fat diets was more nearly equal. It appeared, therefore, that at least part of the tumor-promoting action of fat was due to an accompanying increased consumption of calories (110). Nevertheless, the combined influence of extra calories

and local greasiness may not completely explain the tumor-promoting action of fat, for the incidence of spontaneous breast tumors was increased on high fat diets of controlled caloric intake (197) and for this type of tumor the areas of tumor genesis could not have been influenced by direct contact with fat.

To date all natural fats tested appear to augment the incidence of skin tumors due to pure hydrocarbons (13, 91, 109, 110, 197) with the possible exception of wheat germ oil, which has been reported to be without effect in the incidence of skin tumors due to benzpyrene (85). It is probable, however, that this experiment was complicated by a caloric effect since the authors observed a weight loss in the mice receiving the wheat germ oil. However, not all types of tumors are affected by dietary fat. No effect was observed on the development of sarcomas when benzpyrene, dibenzanthracene or methylcholanthrene were injected subcutaneously into mice or rats (13, 110, 197) and the incidence of the spontaneous lung tumor was likewise unaffected by dietary fat (197).

For still other types of tumors certain fats may actually inhibit carcinogenesis. The presence of 5 per cent of hydrogenated coconut oil greatly retards the production of hepatic tumors in rats fed p-dimethylaminoazobenzene (131). On a synthetic diet containing 5 per cent of corn oil, the incidence of hepatic tumors at 6 months ranged from 53 to 64 per cent, but when the corn oil was replaced by hydrogenated coconut oil, the tumor incidence never exceeded 8 per cent while in most groups it was zero. Since hydrogenated coconut oil is composed almost entirely of saturated fats, it was thought that the presence of an unsaturated fat might be necessary for the genesis of hepatic tumors. However, the inhibiting effect of the hydrogenated coconut oil persisted in rats fed 40 mgm. of ethyl linolate daily. Other differences between the two oils are in chain length and in the amount of antioxidants present.

György and his co-workers (84) have shown that p-dimethylaminoazobenzene was destroyed *in vitro* in diets containing high levels of linoleic acid. Obviously if a fat promotes the destruction of the azo dye in the diet, before it gets a chance to act in the critical carcinogenic areas, no tumors will develop, but the protection of the animal would essentially be a reflection of a reduced intake of carcinogen. Such an explanation, however, could not explain the protective action of hydrogenated coconut oil since the azo dye was shown to be very stable *in vitro* in the diets fed (131).

At least three other fatty extracts have been reported to reduce the carcinogenic effectiveness of the azo dye when fed with it at levels of 5 per cent of the diet or less: rice bran oil (138, 192), an ether extract of yeast (192), and a similar extract of liver (132). "However, the original materials from which these extracts were prepared all happen to be anticarcinogenic by virtue of non-fatty substances such as proteins and B-vitamins which they contain. Analyses of rice bran oil for riboflavin and other B-vitamins revealed the presence of many water-soluble materials (192), although in amounts which were probably insufficient to account for the entire anticarcinogenic effect observed" (131).

A second group of fats appears to have no significant effect on the development of hepatic tumors due to p-dimethylaminoazobenzene. These include cod liver

oil at 1 or 2 per cent of the diet (132), olive oil at 3 per cent (96, 155), cottonseed oil (132), corn oil (94, 132), butter fat (132), wheat germ oil (191) at 5 per cent, and partially hydrogenated vegetable oil (Crisco) at 20 or 30 per cent of the diet (132). These latter oils, however, were usually fed in experiments designed to reveal the effects of non-fatty constituents of the diet, and true differences between the various oils may therefore have been masked. Another complication arises from the fact that as the percentage of fat in the diet increases, the amount of food and therefore of carcinogen consumed by the animal decreases.

When lecithin was fed to rats that were also receiving o-aminoazotoluene, the production of liver tumors was accelerated (4). Apparently, this is the only lipoidal substance reported to augment the development of liver cancers. Cholesterol was without effect (4).

The relationship of cholesterol to tumor development has received the attention of a considerable number of investigators, and the literature has been recently summarized (15). Roffo (167) considers cholesterol of prime importance in the development of tumors due to ultraviolet radiation and has stressed the fact that cholesterol accumulates in certain precancerous lesions in rats and in man. The addition of cholesterol to the diet has been reported to hasten the formation of tar cancer in mice (72). However, there is considerable doubt that cholesterol can be regarded as a universal accelerator of the carcinogenic process. When added to the diet it failed to stimulate the production of tumors by ultraviolet radiation in the rabbit or mouse; Shope virus papillomas developed at essentially the same rate in the controls and in rabbits in which a cholesterolemia had been produced; and the addition of 2 per cent cholesterol to the diet of mice failed to alter the rate of formation, or the number of hepatomas induced by o-aminoazotoluene (4, 13, 15).

Various fats have been reported to have carcinogenic properties when included in the diet. Roffo (168–171) reported that he had induced adenocarcinomas and sarcomas in the stomachs and intestines of rats fed fats or cholesterol heated to 300°C. The minimum period for the production of these lesions was 16 months. Beck and Peacock (18), however, found no lesions of the glandular stomach in rats that had been fed heated cooking fats and oils for a period of 436 days, although papillomas were frequently present. Since the rats fed the heated oils also developed the clinical signs of avitaminosis A, it was concluded that heated fats contained some substance that interfered with vitamin A metabolism. Kirby (98) heated cholesterol at 270 to 300°C. for half an hour in the air, and fed 20 mgm. of the product daily to rats for a period of two years. No significant lesions from the point of view of carcinogenesis were observed in either part of the stomach. Domagk (67) noted tumors or glandular hyperplasia in a few mice that had received a diet of rice plus 20 per cent of olive oil for a year. In one mouse a gastric adenocarcinoma was found and in others the gastric mucosa was the seat of an extensive polypoid overgrowth which probably was precancerous.

Rowntree, Steinberg, Dorrance and Ciccone (175) reported that a large percentage of rats fed a crude wheat germ oil developed sarcomas arising from various areas of the peritoneum. Most of the tumors appeared within 60 to 200

days after the start of the experiment. Several other investigators attempted to confirm these findings but were uniformly unsuccessful (39, 46, 47, 66, 73, 86, 177).

Proteins and amino acids. The importance of cystine for the genesis of several types of neoplastic growths has been stressed by White. Female mice of the C_3H strain were divided into two groups at the time of weaning: one was placed on a diet relatively low in cystine and in total protein while the other group received 0.5 per cent of cystine in addition. The mice on the low cystine diet failed to develop spontaneous mammary tumors, even after 22 months, whereas 97 per cent of the mice on the "high" cystine diet developed tumors (216). Furthermore, the incidence of leukemia induced by methylcholanthrene in mice of the dilute brown strain was reduced when the concentration of cystine in the diet was decreased to such a level that weight increases were prevented. Only 17 per cent of the mice on the low cystine diet developed leukemia as compared to 92 per cent of the controls on a high level of cystine (218). White and Edwards (217) also demonstrated that cystine or methionine could influence the production of hepatic tumors in rats. When rats were fed a low cystine basal diet containing 0.06 per cent p-dimethylaminoazobenzene, 60 per cent developed liver tumors, as compared to 96 per cent in the animals receiving the same diet plus 0.5 per cent of cystine or methionine. White has also shown that the growth rate of a rat can be retarded by the oral administration of methylcholanthrene, benzpyrene or p-dimethylaminoazobenzene (215, 219). This effect was observed only on diets containing high amounts of fat and a borderline level of protein. Growth was restored even in the presence of the carcinogen when l-cystine or dl-methionine were added to the diet. It was suggested that the carcinogens increase the demand on the animal for a sulfur containing amino acid needed for detoxication, and as a result, the synthesis of new tissue protein is prevented (215).

György, Poling, and Goldblatt (83) reported that the combination of cystine and choline retarded liver cirrhosis and hepatomas in rats fed a semi-synthetic diet. This finding however was not confirmed by White (217), and Mori (136) found that 1 gram of cystine per kilo of diet did not inhibit the genesis of liver tumors in rats fed a diet of rice and carrots.

Under certain conditions the development of hepatic tumors induced in rats by the ingestion of p-dimethylaminoazobenzene can be retarded by increasing the amount of protein fed. Kensler and his associates (94) observed that neither casein alone nor riboflavin alone prevented tumor formation, but that the two factors together were very effective. Nakahara and his collaborators (155) reported that 10 per cent of fish protein added to a diet of rice and carrots failed to reduce the incidence of tumors due to the azo dye. Sugiura and Rhoads (192) observed no protection when casein was added to the rice-carrot diet although the nutritional state of their animals was markedly improved by the addition. When suitable B vitamins were given in crystalline form, the incidence of hepatic tumors was essentially the same whether 12 or 18 per cent of casein was fed (133).

Vitamins. In studying the tissue changes that developed in rats as a consequence of vitamin A deficiency Wolbach and Howe (221) were impressed with the greatly augmented growth activity of epithelium. In some animals the number of mitotic figures in the replacing epithelium and the response of the connective tissue and of blood vessels were such as to suggest "the acquisition of neoplastic properties." Other investigators have also observed that a chronic vitamin A deficiency led to changes in some tissues which might be described as preneoplastic. Orton, Burn and Smith (160) noted odontomas on the teeth of rats surviving over a year on a diet low in vitamin A and in a few instances, supernumerary incisor teeth were seen. These odontomas arose from the pulp and consisted chiefly of spindle cells similar to the embryonic cells of the pulp tissue and apparently were not growing very actively. Proliferative stomach lesions have been reported in rats on a diet deficient in vitamin A (78) but such pathological changes can in no way be considered specific since many dietary deficiencies have been shown to induce similar changes (100, 140).

These observations raised the question as to whether a deficiency of vitamin A in the diet would promote the genesis of tumors by a carcinogenic hydrocarbon. To explore this possibility Howe, Elliott and Shear (90) placed one half of a series of rats on a diet rich in vitamin A while the remainder were given a diet deficient in this vitamin. Cholesterol pellets containing 5 per cent of benzpyrene were implanted subcutaneously in all of the animals. If the diet was markedly low in vitamin A the rats died during the latent period of tumor production but when enough carotene was added to permit the rats to survive the latent period, there was no indication of a significant difference in response to this carcinogen.

Of the various members of the B-complex only riboflavin, pyridoxine and biotin have shown any significant influence on tumor genesis. Riboflavin added to the diets of rats fed p-dimethylaminoazobenzene inhibited the formation of hepatomas (94, 132, 133). When a diet containing rice and carrots was fed, the protective action of riboflavin was observed only in combination with a high protein level (94). With synthetic diets, however, riboflavin alone at a level of 10 mgm. per kilogram of ration completely prevented tumor formation up to 6 months, whereas no protection was noted with 0.5 mgm. per kilogram of diet (133). Although riboflavin has a pronounced effect on the prevention of hepatomas in rats fed p-dimethylaminoazobenzene, high levels added to the diet did not retard the growth of established spontaneous mammary tumors in mice, or of the hepatoma 31 transplant in rats. In fact, the growth rate of these tumors was decreased in animals on a riboflavin deficient diet (141). Riboflavin did not prevent the formation of skin tumors due to methylcholanthrene (110).

The incidence of hepatomas induced in rats by p-dimethylaminoazobenzene was greatly decreased when the level of pyridoxine in the diet was reduced to 0.2 mgm. or less per kilogram of ration (133). When pyridoxine was low or omitted entirely from a synthetic ration, less than 7 per cent of the animals developed liver tumors, as compared to an incidence of over 50 per cent on the control diets containing 2.5 mgm. of this vitamin per kilogram. A pyridoxine deficiency has also been shown to retard tumor formation in mice treated with a

solution of 0.2 per cent methylcholanthrene (102). In contrast to the results with epithelial tumors in mice and hepatomas in rats, no very pronounced effects of pyridoxine deficiency were observed on the development of sarcomas induced by the subcutaneous injection of methylcholanthrene (102). It is of interest, however, that the growth of transplantable tumors in both rats and mice is retarded by a diet deficient in pyridoxine (31, 102).

The retarding effect of diets deficient in pyridoxine was independent of caloric intake. In fact, in one experiment in which rats received p-dimethylaminoazobenzene and a low level of pyridoxine, the average daily food consumption was higher and the animals maintained their weights better than in the control group (133). Adult rats can maintain their weights for long periods of time in the absence of dietary pyridoxine (130), but the addition of p-dimethylaminoazobenzene to the diet caused a loss in weight (133).

DuVigneaud and his collaborators (71) have reported that biotin increased the incidence of liver cancers induced by feeding p-dimethylaminoazobenzene. This has been confirmed in part by the observation that egg white in the diet suppressed the formation of liver cancer in rats fed p-dimethylaminoazobenzene (103). Egg white contains an abundant amount of avidin, a substance that renders biotin unavailable as a food factor. West and Woglom (214) did not observe any retardation in the growth of a transplantable tumor in mice fed avidin concentrates. However, these latter workers studied the effect of avidin on the growth of established tumors while the investigations of DuVigneaud (71) and those of Kline et al. (103) were made on the genesis of neoplasms.

Maisin, Pourbaix and Cameran (125) examined the action of riboflavin, vitamin B_1, and nicotinic acid fed to mice on the production of tumors by the application of benzpyrene to the skin. The addition of sodium pyrophosphate plus 20 micrograms of vitamin B_1 daily to the diet of each mouse had no effect on the appearance of papillomas but did delay slightly the malignant change of these tumors. On the other hand, when mice were treated with methylcholanthrene and fed diets containing either an abundant or a poor supply of vitamin B_1, no difference in the rate of tumor development between the two groups was observed (82).

Adamstone (1) fed ferric chloride to developing chicks in order to destroy the vitamin E content in the food. A large proportion of the chicks on this ration developed lymphoblastomata involving the heart, lungs, liver, spleen, pancreas and gizzard. It should be remembered that leukemia in chickens is rather common.

Miscellaneous diets. Various animal tissues are also known to influence carcinogenesis. Maisin and his associates (122) have shown that the inclusion of liver, pancreas or intestinal mucosa in the diet of tarred mice promoted cancer growth whereas brain, thymus, bone marrow, dried gastric mucosa or dried lymph nodes inhibited the development of cancer induced by tar. Growth-inhibiting and growth-promoting factors were often found in the same organ and, in general, substances which augment tumor genesis were water soluble at pH 7–8.4 and were relatively insoluble in ether, whereas the inhibiting fractions are soluble in

ether and are relatively insoluble in acetone (122). An acceleration of skin tumors following applications of tar or benzpyrene was observed in mice that received whole liver in their diets (13, 211). However, the feeding of liver did not augment the formation of tumors due to ultraviolet radiation or to the injection of hydrocarbons (13, 137).

The most striking effect of dietary liver is observed on the development of liver tumors induced by the azo dyes. When 10 per cent dried whole liver is included in the diet of rats fed or injected intraperitoneally with p-dimethylaminoazobenzene, the formation of liver cancer is greatly retarded (132, 135, 137, 154, 155). An alcohol liver fraction, i.e., that portion of the water soluble material of whole liver that is insoluble in 70 per cent alcohol gave good inhibition when fed at a level of 2 per cent (132). In a search for other animal tissues which might likewise inhibit the formation of liver cancer, Mori (135) found that kidney was also effective but spleen, muscle, brain, lung, stomach, intestine, testicle and bile had no effect. The pancreas even stimulated tumor formation to a mild degree. Apparently the retarding effect of liver is limited to the genesis of hepatomas since liver feeding did not protect against the development of sarcomas in strain C mice induced by the subcutaneous injection of o-aminoazotoluene (203).

An antiblastogenic factor has been found in fresh beef heart. The incidence of tumors in mice painted with a solution of methylcholanthrene was reduced from 42 per cent in the controls to 27 per cent in the group that were fed fresh beef heart. Drying the heart tissue 24 hours at 70 to 90° destroyed the factors responsible for the inhibition (123).

The retarding influence of yeast on the genesis of tumors has also been reported. Nakahara, Fujiwara and Mori (153) observed a reduction in the number of hepatomas in rats given weekly intraperitoneal injections of p-dimethylaminoazobenzene and fed dried baker's yeast to the amount of 15 per cent. Between the 257th and 279th day liver cancer was found in 3 of the 6 survivors of the control group as compared to no tumors in 10 survivors of the rats fed yeast. Other investigators (132, 193) have also shown that hepatomas induced in rats by the ingestion of p-dimethylaminoazobenzene could be inhibited by including at least 15 per cent yeast in the diet. Yeast heated to 80°C. for 7 minutes was found to decrease the incidence of skin tumors in mice due to benzpyrene (124).

A decrease in the rate of formation of hepatomas due to azo dyes has been noted when rats were fed various grain diets. Thus when wheat (9, 77, 97, 206), rye (120), or millet (139) was used as a basal diet instead of rice, the incidence of liver tumors was reduced. On the other hand, a water soluble rice bran concentrate has been shown to augment the genesis of liver tumors induced by p-dimethylaminoazobenzene (132). This concentrate is an excellent source of the known vitamins of the B complex with the exception of riboflavin. It should be recalled that synthetic diets low in riboflavin but with an abundant supply of the other vitamins of the B-complex have been shown to augment the formation of liver tumors (133).

B. *Miscellaneous.* The influence of various hormones on tumor genesis has

been the subject of a considerable number of investigations and most of these have been concerned with the effect of the sex hormones. This information has been summarized in previous reviews (3, 81, 106, 115, 142).

It is generally accepted that in experimental mice the tendency to develop spontaneous mammary tumors is controlled by inheritance, hormonal stimulation and by a third factor which is usually transferred by nursing. The significance of heredity in this phenomenon has been summarized by Loeb (114). Recently the importance of the factor transmitted by the milk has been clearly demonstrated and the results of the investigations have been summarized by Bittner (34). There is now some evidence that the agent responsible for this effect is a protein of a size comparable to that observed for some of the viruses, and the suggestion has been made that the factor might indeed be a virus (33). The agent has also been demonstrated in the spleen, blood, and lactating mammary tissues as well as in the milk (8, 222), and there is some evidence that it might also exert an effect on embryos *in utero* (74). Furthermore, it has been reported that the growth of some types of mouse leukemia was also influenced by foster nursing although this was not true for all strains (99, 112).

Turner (202) noted that a growth-inhibiting substance derived from human urine prevented, to a limited degree, the formation of tumors in mice following the injection of dibenzanthracene or methylcholanthrene. Of 54 mice injected with either of these hydrocarbons, followed by daily injections of the inhibitor, 30 per cent developed tumors as compared to 80 per cent of the controls not treated with the extract. The urinary derivative had little or no effect on transplanted or spontaneous tumors in mice.

In a number of papers Maisin and his collaborators (104, 126) have reported that certain organic peroxides altered cancer incidence in mice treated with benzpyrene. Since tumor tissue has a greater anaerobic glycolysis than corresponding normal tissues, it was thought that the use of substances which readily yield free oxygen might retard tumor genesis. These investigators injected these "peroxides" either in aqueous solution or in the form of crystals, and reported that there was a different effect on cancer incidence depending upon the concentrations used. Strong concentrations, i.e., up to 1×10^{-3}, had an activating effect, hastening the appearance and increasing the incidence of malignant growths. Beginning with a dilution of 1×10^{-5} an inhibitory effect was observed and this inhibition increased up to a dilution of 1×10^{-18}. In a comprehensive series of experiments Belkin (20–22) was unable to confirm these results. The latter investigator tested the effect of the peroxide on the incidence and growth of transplantable, induced, and spontaneous mouse tumors, using a very large number of animals in his series. The results were uniformly negative in all cases.

SUMMARY

The extrinsic factors that alter the action of carcinogenic agents include various chemical and physical agents such as fats, oils, "irritating" substances, trauma and radiant energy all of which have a pronounced influence on tumor

formation when applied directly to the tissues undergoing carcinogenic changes. Other factors are active when administered at a distance from the tissues undergoing neoplastic conversion. These include alterations in the diet involving calories, fats, proteins and amino acids, vitamins, and modifications in the balance of certain hormones.

It should be stressed that altering the carcinogenic process does not necessarily imply a similar effect on the growth of tumors. The investigations cited illustrate the extreme caution necessary in the interpretation of results observed. Factors that influence tumor growth might be obscured when overwhelming doses of carcinogen are employed, and any treatment that interferes with the dietary intake or with the general health of the animal may lead to false conclusions.

The ultimate aim of experiments of this nature is a better understanding of the mechanism of neoplastic development. Minor successes have been attained in this direction, and the suggestion has been made that carcinogenesis progresses not as a single process but rather in two or more distinct stages: *a*, the preneoplastic stage or the latent period of carcinogenesis, and *b*, the neoplastic stage. Various agents have been shown to act on one or the other period. In general, however, the experiments raise more questions than they answer. Thus, the nature of the effect due to fat, to croton resin, to calories, or to the factors involved following injury, as well as the various effects of dietary constituents all must await further experimentation.

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DISTRIBUTION OF VITAMIN A IN TISSUE AS VISUALIZED BY FLUORESCENCE MICROSCOPY

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Attempts to localize biologically active substances in tissues by histo-chemical and histo-physical methods have been carried out repeatedly in order to clarify their rôle in the organism (1, 2). The demonstration of vitamin A by a histologic method seems to be important in view of its still problematic rôle in the body.

Vitamin A lends itself to morphologic demonstration because its fat solubility prevents its solution in aqueous fixatives. There have been some attempts to visualize it by histo-chemical methods, but these were of questionable specificity (3, 4, 5). In recent years the fluoresence of vitamin A in ultra-violet light has been used for its demonstration in tissues. This review presents the findings obtained by this method.

HISTORICAL INTRODUCTION. In 1932, Von Querner (6) described a luminescent substance (Leuchtstoff X) in isotropic fat droplets in the liver cells. Its strong fluorescence was quickly destroyed by the ultra-violet light eliciting this phenomenon. In further studies he (7) found this substance soluble in lipoid solvents and not in aqueous solutions. After various attempts to identify it by histo-chemical examinations, he (8) finally concluded that it is related to pigments, probably the fat soluble carotenoids. Since Querner encountered a similar fluorescence in fish liver oils and in vitamin A concentrates, he assumed that the luminescent substance found in liver, retina, adrenal cortex and pituitary body is vitamin A.

Querner deserves the credit for having pointed to the vitamin A fluorescence as a means of demonstrating vitamin A in tissues even though his histological descriptions were incorrect and the few animal experiments reported were insufficiently controlled. Prior to Querner, Hirt (9) described bright yellow fluorescent droplets in the wall of the liver sinusolds in living animals which he (10) later related to vitamin A. Querner localized the fluorescence in the lipoid droplets of the epithelial liver cells, whereas Hirt and his co-workers found them in the Kupffer cells. In a more recent, chiefly vital, microscopic study Hirt and Wimmer (11) found the greatest amount of vitamin A fluorescence in the Kupffer cells of the liver and endothelial cells of the lung, less in the endothelial cells of other organs. The parenchymatous cells were reported as free of vitamin A fluorescence. In addition, they found in living organs different fluorescent substances which they associated with various members of the vitamin B complex and different combinations of them with proteins, with vitamin C, or with the active liver principle of pernicious anemia. Their reports, so far accessible, do not give sufficient information as to how these different fluorescent details in the living organs may be differentiated and fail to offer con-

trolled studies as to the specificity of the fluorescent phenomena. As long as there is not more known about the basis for their assumption, Ellinger's (12) criticism against their theories appears justified. Nevertheless, their observations in living organs are important for the evaluation of the somewhat clearer pictures of fixed specimens. Far better substantiated appear the observations of Jancsó and Jancsó (13) who observed an increase of the characteristic fluorescence of the liver following the administration of carotene and vitamin A, and also a green fading fluorescence in the pigment layer of the retina, depending on the degree of light adaptation. The recent German investigations of Schairer, Rechenberger, Gockel and Patzelt (14), Patzelt and Schairer (15), and Schairer and Patzelt (16) are accurate and well controlled studies of vitamin A fluorescence. Their observations appeared simultaneously with those of our group in this country. Since the studies were obviously independent they confirmed each other. Their reports and the more extensive ones of our own group represent the basis of the following presentation.

A METHOD OF HISTOLOGICAL DETERMINATION OF VITAMIN A. *1. Physical Principle.* Vitamin A has a maximum absorption in the higher ultra-violet range at 328mμ (17). If exposed to ultra-violet light of similar wave length, vitamin A assumes a bright green, rather quickly fading fluorescence (18, 19). With given solvent and illumination, the fluorescence is the brighter and the fading the slower the higher the concentration of vitamin A. If the illumination is reduced, *e.g.*, by screening out some of the light, the fluorescence is duller, but the fading protracted. Similarly, in certain solvents equal amounts of vitamin A fluoresce more brightly and fade faster than in others (20).

2. Principle of Fluorescence Microscopy. The visible picture is observed which is produced if ultra-violet rays strike fluorescent structures in the examined specimen. These structures change the ultra-violet light which they absorb to visible light of different wave length. The remaining ultra-violet rays are absorbed by a filter in the eye piece (*e.g.*, Leitz ultra-violet protecting filter no. 8574-A).

Various applications of fluorescence microscopy in technical and biological sciences have been reported (21, 22, 12). In biology, either living objects, usually in incidental light, (vital-microscopy, see Ellinger 12, Singer 23) or specimens, usually in transmitted light, have been studied. The fluorescence microscopic picture of human organs has been described in detail (Hamperl 24. Sutro 25, and others). Furthermore, staining with fluorescent dyes (fluorochromy) has been applied (22).

3. Fixing. Fixation in solution of formaldehyde U.S.P. 1:10, up to eight hours in animal organs, up to eighteen hours in human organs, does not affect the vitamin A fluorescence. The sections are mounted in water and examined immediately (14, 26). The common mounting media for frozen sections either yield a disturbing fluorescence (glycerine) or dissolve vitamin A (mineral oil).

4. Microscope. The light of a mercury vapor bulb is filtered through an ultra-violet filter (such as the Corning glass color filter no. 584) to eliminate the visible light. The residue of red rays is absorbed by a glass cell containing

a 5 per cent cupric sulfate solution or an additional Corning glass color filter no. 428. Since the vitamin A fluorescence in tissue sections is produced by rays in the longer ranges of the ultra-violet which are not absorbed by glass, a standard microscope with a standard Abbe condenser is used (27, 15).

5. Observation. Under the fluorescence microscope a dark field is seen in which fluorescent material stands out. Nearly all tissue constituents reveal some kind of fluorescence. This permits orientation in an unstained tissue section. Vitamin A fluorescence is considered any fading fluorescence of green hue in contrast to other types of green fluorescence which are ultra-violet stable.

6. Localization of the Fluorescence. In frozen sections stained with hematoxylin or methylene blue the vitamin A fluorescence is visible (7, 26). If the ultra-violet filter is changed to a ground glass filter the sections can be studied in visible light and the fluorescence localized. For the study of fat distribution, sections were examined after treatment for three minutes with a 0.1 per cent aqueous phosphin 3R solution. By this method fats reveal a silver-white fluorescence on a brown background. This procedure permits more and finer droplets to become visible than is possible with the routine fat methods, because of the water solubility of the dye (27).

7. Photography. Vitamin A fluorescence is photographed by the use of a 35 mm. camera with a periscope view finder on the microscope attachment. The film material should be either of maximal daylight sensitivity or sensitized for green and yellow (28).

EVIDENCE FOR THE SPECIFICITY OF THE VITAMIN A FLUORESCENCE. Several points of evidence which are partly suggestive and partly conclusive, support the belief that the green, quickly fading fluorescence in tissue sections is due to the presence of vitamin A.

1. Suggestive Evidence (as to the fluorescence in human and animal organs). A. *Visual appearance of the fluorescence.* The green fading fluorescence of adrenal and liver sections stimulates under the microscope the fluorescence of droplets of an oil-in-water emulsion of oils containing vitamin A, such as percomorph or halibut liver oils (8), various commercially available vitamin A concentrates or solutions in corn oil of vitamin A alcohol, vitamin A acetate or vitamin A palmitate (27). Attempts to supplement the visual observation by spectroscopic analysis of the green fluorescence in tissue sections, by Querner (8), Schairer and his co-workers (14) and ourselves, did not succeed because of the quick fading of the fluorescence. However, if the light which excites the fluorescence, is roughly analyzed by the use of glass filters, the rays producing the green, fading fluorescence in tissues are localized between 310 and 350 mμ, that is, in the neighborhood of 328 mμ, the specific absorption maximum of vitamin A.

Carotene imparts under the microscope a dull, very slowly fading green fluorescence which is visible only in higher concentrations and which is easily differentiated from the vitamin A fluorescence (14, 26).

The biologically inactive anhydro ("cyclized") vitamin A (29, 17) gives chemical reactions similar to those of vitamin A, but imparts not the charac-

teristic green, quickly fading fluorescence of vitamin A, but a dark brown fluorescence which slowly changes to a duller green and then slowly fades.

Corn oil and cotton seed oil do not show vitamin A fluorescence. The following fat soluble biologic substances dissolved in corn oil as tested under the fluorescence microscope in an oil-in-water emulsion do not show a green, quickly fading fluorescence: Several vitamin D preparations, alpha- and mixed tocopherol, synthetic vitamin K (2 methyl-3-phytyl-1-4 napthoquinone), estrone, alpha-estradiol, testosterone, progesterone, desoxycorticosterone, cholesterol, cholesterol esters and 1,2,5,6 dibenzanthracene.

B. *Histo-chemical analysis.* The green, fading fluorescence in the tissue sections is abolished by reagents which either dissolve or destroy vitamin A and is not changed by reagents which have no influence upon vitamin A (7, 14, 26).

C. *The distribution of vitamin A fluorescence.* Its distribution in animal or human organs agrees with that found by chemical analysis using the antimony trichloride (Carr-Price, 30) reaction, by spectroscopic analysis, and by biological assay as far as they are available (31, 32,33, 34).

D. *The relation of the characteristic fluorescence to the vitamin A metabolism in human beings.* In a large series of human livers obtained at biopsy or autopsy, a fairly good agreement has been found between the amount of vitamin A fluorescence and the concentration of vitamin A as determined chemically (35), this partly in contrast to the findings of Schairer *et al.* (14) who considered the existence of a type of vitamin A, which is chemically demonstrable, but not fluorescent in tissues. Experience permits one to predict the result of the chemical analysis on the basis of the histologic picture. In diseases associated with prolonged malnutrition or in other conditions in which the chemical analysis yielded traces of vitamin A, usually very small amounts of vitamin A fluorescence were found (27). After feeding of high doses of vitamin A before operations, very high amounts of vitamin A have been found in the biopsy specimens obtained during the operation (36, 35).

E. *The characteristic fluorescence in deficiencies other than vitamin A deficiency.* In rats deficient in vitamin B_1, B_2 and D, as well as in guinea pigs deficient in vitamin C, a normal distribution of the vitamin A fluorescence was seen (37).

2. Conclusive Evidence as to the Fluorescence in Organs of Rats. A. *Depletion experiments.* While rats are kept on a vitamin A deficient diet, the green, fading fluorescence in liver, adrenal, ovary and lung gradually disappears (14, 15) parallel with a decrease of the liver vitamin A as demonstrated chemically (38, 39). The fatty deposits in the livers of vitamin A deficient rats intoxicated with carbon tetrachloride did not show vitamin A fluorescence: fat appearing in the liver reveals the green, fading fluorescence only if vitamin A is available (40).

B. *Repletion experiments.* If vitamin A is fed to rats depleted of vitamin A, a green, fading fluorescence reappears in typical distribution (14, 15) and the amount of this fluorescence found agrees with the amount of vitamin A fed and with the amount found chemically in the liver (38). If much vitamin A is fed over a prolonged period, parallel with an extreme increase of the vitamin A concentration, a very striking vitamin A fluorescence appears in organs normally

showing moderate fluorescence and some in organs normally free of vitamin A (11, 13, 14, 38, 39). If carotene is fed, after a somewhat longer interval vitamin A fluorescence reappears (15, 38), again parallel to the amounts of carotene given and to the vitamin A content of the organ. Feeding of other substances such as different oils or vitamins failed to produce the characteristic green fluorescence in the organs of vitamin A deficient rats (38).

It was important to rule out the possibility that the green, fading fluorescence in tissue sections is due to riboflavin which, dissolved in the aqueous phase of a water in oil emulsion, shows such a fluorescence. Indeed, Hirt and Wimmer (41, 11) considered a part of the green, fading fluorescence seen in living tissues to be due to vitamin B_2. However, the green, fading fluorescence in fixed tissues is dependent upon the alimentary intake of vitamin A and independent of the intake of B_2, and is not affected by reducing agents (14, 38). Possibly, the water soluble riboflavin disappears from the tissues during fixation and preparation of the sections (38).

Summarizing, conclusive evidence exists that the green, fading fluorescence in sections of rat organs is due to vitamin A. For human organs only suggestive evidence is available. Consequently, in describing the green, fading fluorescence in rats one is justified in calling it vitamin A. In other animals or in human organs, one should speak of vitamin A fluorescence, which indicates that it can be caused by vitamin A but does not exclude other, so far unknown, substances.

STABILITY OF THE VITAMIN A FLUORESCENCE. Since vitamin A is quickly destroyed by exposure to air or oxidizing agents (42) the vitamin A fluorescence disappears from frozen sections within a few hours. The speed of disappearance is greater in animal than human organs with variations between different organs. It vanishes much faster from the Kupffer cells than from the epithelial cells of the liver, although the fat distribution remains unchanged. It disappears much faster from tissue sections kept in water than from those kept in plasma, serum, or liver emulsion. This indicates the presence of a protective principle. This principle which in smaller amounts is found in urine, bile and organ emulsions is thermostable, not ether soluble and is more effective at 37° than at room temperature. Its biologic significance is still problematic (43).

Roentgen irradiation does not decrease the vitamin A fluorescence (44), contrary to what Querner (45) reported.

INFLUENCE OF CARRIER SUBSTANCES ON VITAMIN A FLUORESCENCE. As in non-biological solvents, the vitamin A fluorescence in tissue varies in character, depending on the carrier for vitamin A. In larger fat droplets it is usually less bright, but more slowly fading than in smaller ones. Another factor is the presence of pigments which screen the ultra-violet rays out, thus reducing the brightness, but retarding the fading of the fluorescence. The presence of carotene in the human liver explains why the fluorescence is more slowly fading but duller than carotene-free rat livers. These variations in brightness with equal amounts of vitamin A prevent an exact quantitative determination of vitamin A in tissues by fluorescence microscopy.

Relation Between Fluorescence and Vitamin A Concentration. Vitamin A

fluorescence is visible in droplets of an oil-in-water emulsion if the oil contains more than 20 micrograms of vitamin A per gram, whereas the dull carotene fluorescence is revealed by oils containing more than 150 micrograms per gram. The vitamin A concentration of the individual fluorescent detail in the tissue is above this level. In liver, containing more than 1.5 micrograms of vitamin A per gram, vitamin A fluorescence is encountered (38). In organs in which only certain structures contain vitamin A, the histological method is far superior to the chemical analysis.

VITAMIN A DISTRIBUTION IN DIFFERENT ORGANS UNDER NORMAL AND PATHOLOGICAL CONDITIONS. *Liver.* The liver is the chief depot of the body for vitamin A (31, 32). In the livers of human beings, rats, rabbits, monkeys, dogs, guinea pigs and frogs, vitamin A fluorescence has been described in several sites (14, 26, 38, 46).

I. *In the Kupffer cells.* The fine fat droplets which normally fill the cytoplasm of the Kupffer cells (47) reveal vitamin A fluorescence, except in nutritional deficiency (38). Their absence is uncommon but not necessarily pathologic.

II. *In the epithelial cells.* (a) Fine fat droplets occur near the borders adjacent to the sinusoids. These droplets, demonstrated with sensitive fat stains, are arranged like a string of beads. If large amounts of vitamin A are present those droplets reveal marked fluorescence. They may enlarge and then several of them fill the cytoplasm. (b) Large fat drops, demonstrated by routine fat stains. One of them fills the cytoplasm and usually pushes the nucleus to the side. Their vitamin A fluorescence is very variable and usually lower than that of the small droplets, even in livers containing much vitamin A. (c) Fine bars or droplets, irregularly aggregated throughout the cytoplasm and only sometimes lining up on the edge. Their quickly fading fluorescence is hardly recognized under low magnification; they are not demonstrable by phosphin 3R. Their appearance simulates that of mitochondria (48). That mitochondria contain vitamin A has been proven chemically (49) and suggested by histo-chemical studies (4, 5). Mitochondria of germ cells contain also carotenoid pigments (50). (d) Lipofuscin (wear and tear pigment), which has been considered as a protein compound colored by carotene (51). If the granules show fat reaction and vitamin A fluorescence their red-brown fluorescence appears only after the latter has faded. (e) The cytoplasm imparts a diffuse green, fading fluorescence which is removed by alcohol. It seems to be due to small amounts of vitamin A diffusely spread over the cytoplasm.

The different sites of vitamin A fluorescence in the liver cause a great variability of the picture with and without relation to the lobular topography. The significance of this polymorphic distribution is as yet unexplained and only a few points can be gathered from experiments on animals under controlled conditions.

I. *Influence of Various Experimental Conditions Upon the Vitamin A Distribution in the Rat Liver.* A. *Influence of diet.* 1. *Hypervitaminosis A.* Livers of rats, which had received large doses of vitamin A for several weeks and showed the signs of hypervitaminosis A (52, 53, 54, 55), reveal an extremely strong

vitamin A fluorescence. The enlarged Kupffer cells are very strongly fluorescent and rich in fat (54, 56), whereas the epithelial cells show the usual fluorescence, chiefly imparted by fine fat droplets and also by the cytoplasm. Around the periportal and central veins, fine fluorescent fat droplets appear in endothelial cells and other connective tissue cells (38, 39).

II. *Unbalanced Nutrition.* Rats kept for more than three weeks on otherwise balanced diets consisting predominantly of protein, carbohydrate or fat respectively (see 57), reveal characteristic variations partly paralleling that of the fat distribution (58). The rats on a high protein diet show less vitamin A than the others, much of it localized in Kupffer cells; in the lobular centers many mitochondria are seen in the epithelial cells, whereas in the periphery fine droplets line their edge. In the animals fed much fat, most of the vitamin A is localized in medium-sized fat droplets on the edge of the liver cells, less in Kupffer cells and some in isolated large fat droplets on the periphery. In those given much carbohydrate the most vitamin A, but also the most morphologically demonstrable fat (59), was seen; the strongly fluorescent fat droplets near the border of the cells increased in size towards the periphery of the lobule.

In starving rats, the chemical vitamin A concentration does not significantly change (60) despite marked microscopic alterations in fluorescence. The vitamin A fluorescence becomes irregular, and moderately fluorescent large fat droplets accumulate at the lobular periphery. When in later stages no fat is morphologically demonstrable, the cytoplasm imparts a strong green, fading fluorescence indicating that the latter carries most of the vitamin A instead of the visible fats (58).

III. *Choline Deficiency.* In rats on choline deficient diets (supplemented by carotene), in which fatty livers develop (61), the vitamin A fluorescence of the fat droplets in the liver markedly decreases. First, irregular foci remain in which Kupffer cells and fat droplets in the surrounding liver cells impart the fluorescence; later, only a few isolated fat droplets reveal some vitamin A (62). If vitamin A instead of carotene is fed, the Kupffer cells maintain vitamin A, whereas the liver cell fat is devoid of it. It appears that choline or related substances (labile methyl groups) are connected with the presence of vitamin A in the fat of the liver cells. If additional studies corroborate the impression that in choline deficiency vitamin A storage in the Kupffer cells occurs only after vitamin A and not after carotene feeding, it would indicate that only the liver cells are able to split vitamin A into carotene, something they are unable to do in a fatty liver. It has been repeatedly claimed that liver damage interferes with the conversion of carotene into vitamin A (63, 64, 65, 66 and others).

B. *Experimental Liver Damage.* I. *Intoxication with Hepato-Toxic Drugs.* Intoxication with drugs with possible hepato-toxic effect, as phosphorus, bile acids, thyroxin, phenolphthalein and sulfanilamide, causes a shift of the vitamin A fluorescence in the liver (40) without necessarily reducing the hepatic vitamin A concentration (63, 67, 68, 69, 70). Within one day after administration of carbon tetrachloride (40) the fluorescent small fat droplets near the border of the liver cells enlarge in the lobular center which leads to an accumulation of

strongly fluorescent medium-sized droplets. Following repeated administration, the vitamin A disappears from the Kupffer and liver cells in the uninvolved peripheral area and is seen only in the central fatty areas. After more than one month, only around the central area a few large fat droplets and some histiocytes reveal vitamin A fluorescence, whereas fatty areas in the intermediary and even peripheral zone are no longer fluorescent; the chemical concentration in this stage is reduced (see also Haig and Post, 71). If the rats receive high vitamin A supplements, the vitamin A fluorescence in the fatty area is more marked and the Kupffer and liver cells in the uninvolved area retain it longer. With a high carbohydrate diet the fatty changes are less marked than with high fat or protein diet and the normal Kupffer and liver cells keep vitamin A longer. During recovery from this intoxication, rats on a high protein diet show vitamin A in the Kupffer and liver cells of the uninvolved areas before rats fed much fat or carbohydrate do (faster regenerations).

II. *Cirrhosis.* In animals with experimental dietary cirrhosis as produced by György and Goldblatt (72), the vitamin A distribution is the more irregular the farther advanced the liver damage. In early stages a non-lipid soluble material with non-fading, gold-brown fluorescence is seen in liver cells and the adjacent Kupffer cells. Later it accumulates in connective tissue cells of the proliferated periportal spaces (73). This material, which is fluorescent even in paraffin sections, is apparently a break down product of fats. It seems identical with ceroid, described by Goldblatt and György (72) and Lillie et al. (74).

III. *Carcinogens.* Some carcinogenic hydrocarbons reduce the vitamin A stores in the liver (75, 76, 77, 78), especially that of the mitochondria (49). However, in the recovery a normal fluorescence microscopic picture is seen (Marron, 79).

C. *Depletion of Vitamin A Depots.* Rats put on a vitamin A deficient diet immediately after weaning lose their small vitamin A stores within 22 days, before their weight becomes stationary or clinical signs of vitamin A deficiency appear (15, 38). The fluorescence of the cytoplasm disappears first, then that of the fine droplets near the border of the liver cells, and finally that of the Kupffer cells; fat droplets retain it the longest. If rats are put on the vitamin A deficient diet a few days after weaning their depletion is retarded (see 80). Adult stock animals need about six months for depletion. Albino rats are more quickly depleted than pigmented ones (16).

If rats are first made hypervitaminotic and then put on a vitamin A deficient diet (39) fluorescence of the Kupffer cells decreases first; that of the liver cells remains. The marked preponderance of the fluorescence of the Kupffer cells gradually subsides, after the vitamin A concentration drops to a third of that in the hypervitaminotic stage. The decrease of the Kupffer and liver cell fluorescence is then parallel. In advanced hypovitaminosis droplets are found in the epithelial cells very close to the Kupffer cells and once more a preponderance in the Kupffer cells appears. Finally, a few Kupffer cells show the last remnants of fluorescence. Male rats are depleted faster than female rats. The serum vitamin A, which was higher in males than in females, was not related to the fluorescence. These experiments indicate that the Kupffer cells not only

distribute vitamin A but also destroy it. They apparently cause the rapid vitamin A loss in hypervitaminotic animals on a vitamin A deficient diet (81). Whether this non-economic utilization is related to a deficiency in vitamin E (82) remains to be examined.

D. *Repletion of Vitamin A Depot.* After feeding of more than 250 units of vitamin A to rats, the liver of which is free of vitamin A for a few days, the fluorescence reappears (15), first in the Kupffer cells and then in small droplets in the adjacent part of the liver cells. The speed of reappearance depends on the amount fed (38). In animals depleted for more than a week the repletion is irregular, apparently due to disturbance of intestinal absorption. The results of feeding carotene are variable and the fluorescence appears later and in smaller amount; before the appearance of vitamin A fluorescence, the epithelial cells of the liver reveal a diffuse, faint, green, slowly fading fluorescence, probably due to the temporary presence of carotene (15, 38) For replacement of vitamin A by subcutaneous or intraperitoneal administration, larger doses and a longer interval are required (see 83, 84, and others). Carotene administered parenterally did not restore the vitamin A fluorescence (15, 38). After feeding of vitamin or carotene to rats, intoxicated by carbon tetrachloride, the fluorescence appears in the large fat droplets of the liver and only with large doses in the unaltered areas. This indicates a higher avidity of the fatty areas for vitamin A (40).

2. Variations in the Distribution of Vitamin A Fluorescence in the Human Liver. The marked variations encountered were not caused by postmortal changes since specimens of livers obtained for biopsy during laparotomy reveal similar pictures (36). Certain findings were characteristic even in respect to the marked polymorphism (26, 85, 35, 86).

A. *Fat Deposition.* Localized or generalized infiltrations of medium-sized and large fat droplets may show absence of, moderate, or strong, vitamin A fluorescence. In alcoholics, the picture often resembles that of the choline-deficient fat liver (62). This raises the question whether similar dietary factors may not decide the vitamin A fluorescence of the large fat droplets in the human liver.

B. *Preponderance in Kupffer Cells.* In the presence of huge amounts of vitamin A, *e. g.*, after feeding of large doses, most of the fluorescence is in the Kupffer cells and the liver cells have a normal appearance. However, in some conditions, like diabetes mellitus, uremia, and obstructive jaundice, the Kupffer cells reveal strong fluorescence, whereas the liver cells are almost devoid of it. In visible light these areas show usually a toxic edema. Since in diabetes clinical signs of vitamin A deficiency occur (87), possibly the lack of vitamin A in the liver cells with accumulation in the Kupffer cells interferes with its utilization.

C. *Focal Parenchymal Damage.* The vitamin A fluorescence may either disappear from the area involved as in recent thrombosis of the portal vein or with necrosis of the lobular center, or fluorescent material may accumulate in peculiarly shaped sites as in irregular fat droplets in the liver cells or in bizarre shaped Kupffer cells.

D. *Hepatitis.* The greatest polymorphism occurs in acute hepatitis. In

acute yellow atrophy very much vitamin A fluorescence may be imparted by irregular shaped fat droplets in liver cells; all visible fat is fluorescent. In subacute atrophy less fluorescence is seen and some livers are almost devoid of it. In atrophy with transition into cirrhosis, histiocytes and endothelial cells in the vascular connective tissue may be rich in vitamin A fluorescence (see chemical study of Cox 88).

E. *Liver Cirrhosis.* In arrested forms, the lobules and regenerated pseudolobules reveal a nearly normal distribution. Fatty areas may show much or little vitamin A fluorescence. Connective tissue cells in proliferated periportal fields often contain fine fat droplets with fluorescence. In progressive cirrhosis, especially with jaundice, only scattered droplets in liver cells or some bizarre shaped Kupffer cells reveal vitamin A fluorescence. The decreased fluorescence, often despite high fat content, parallels the low chemical vitamin A concentration (89, 90, 91, 92, 64).

F. *Irregular Distribution.* In addition to the described alterations others occur which are less easily classified. The fluorescence pattern may vary from cell to cell or from cell group to cell group without difference of the histological picture in visible light. The irregularity of the vitamin A distribution is not mirrored by the chemical analysis. Marked degrees of disturbance of the vitamin A fluorescent pattern are associated with parenchymatous damage upon routine histological analysis or clinical study of the liver function (85). Consequently, milder degrees of this disturbance are considered as initial liver damage not recognized by the common routine histological methods (35, 36). According to this standard and in comparison with normal experimental animals almost no human liver available for examination is entirely normal.

G. *Age Influence.* In the fetus much vitamin A fluorescence is found in liver and Kupffer cells. Toward term it markedly decreases and in new born infants only traces are seen. In the second month, an increase starts and after the second year the vitamin A distribution approaches that of the adult (26). These findings agree with chemical analyses (93, 94, 95, 96).

H. *Estimation of the Total Amount of Vitamin A Fluorescence.* The total amount of fluorescence was low, in primary liver disease, in obstructive jaundice, malnutrition resulting from tumor and also in renal conditions; this agrees with chemical analyses (92, 95, 97 and others).

3. Significance of the Vitamin A Distribution in the Human Liver. A few points can be made so far from the study of vitamin A fluorescence.

A. *The Rôle of the Kupffer Cells.* The rôle of the Kupffer cells in fat (98) and vitamin metabolism (11), especially in that of vitamin A (99), has been stressed. The Kupffer cells are the last to be depleted and the first to be repleted. They absorb vitamin A from the blood and transmit it to the liver cells, as seen from its presence in the portions of the liver cells adjacent to the Kupffer cells; a block in this transmission is probably responsible for the picture in toxic edema. They contain the excess of vitamin A in hypervitaminosis and probably destroy it (46). They store carotene if it is injected in aqueous solution (66). After blockade of the Kupffer cells the storage of vitamin A in the liver is interfered with (100, 68, 101).

B. *The Rôle of the Liver Cells.* The liver cells are the permanent depots for vitamin A. In liver damage they may be unable to hold it or release it to the blood. From large fat droplets vitamin A is very slowly released (46).

C. *Relation Between Plasma Vitamin A Concentration and Distribution, and Amount of Vitamin A Fluorescence* (35). Comparison of the plasma vitamin A level with hepatic vitamin A concentration and fluorescence, in specimens of liver removed for biopsy, over a wide range of the plasma vitamin A level shows a parallelism only if an average of a number of cases is taken. In individual cases no definite relation between plasma level and liver stores exists. High plasma level usually indicates normal or high liver stores, whereas low levels may be associated with high or low liver concentration. The plasma level runs somewhat parallel to the vitamin A fluorescence of the Kupffer cells, less so to that of the epithelial cells. With marked discrepancy between plasma level and hepatic vitamin A concentration the vitamin A pattern is very much disturbed, and liver function is impaired.

D. *Vitamin A Shift in the Liver as a Cause of Functional Avitaminosis.* The shift of the vitamin A from normal to pathologic sites in the liver may explain the drop of the plasma vitamin A level found in acute liver diseases or in pneumonia. (Literature, see 102.) A lack of vitamin A intake cannot explain this phenomenon since months are required to reduce the plasma vitamin A level of healthy adults by a vitamin A deficient diet (103, 104, 105). Apparently, the liver regulates the plasma vitamin A level and that explains the parallelism between liver stores and blood level in healthy animals (106, 107, 108). In liver damage in which vitamin A has shifted to places where it is not available for immediate release, the plasma vitamin A level may drop in the presence of quantitatively sufficient liver stores and a functional avitaminosis exist due to disturbed release (109, 97, 35). In healthy young rats clinical signs of vitamin A deficiency appear only a long time after vitamin A has been discarded from the liver, whereas in human beings the liver is never completely free of vitamin A even if clinical signs of vitamin A deficiency appear, such as hemeralopia as seen in liver diseases (110, 111, and others).

The value of vitamin A therapy in those cases is apparent from the histologic picture (112). Large doses have to be given in order first to saturate the pathologic sites which have an increased avidity for vitamin A and then to enforce storage in the normal sites from which vitamin A is more readily available for use. Actually, giving large doses to carbon tetrachloride intoxicated rats (40), or human beings with liver damage (38) resulted in storage of vitamin A in normal sites.

4. Fish Liver. In fish livers (113) fluorescence is imparted by medium sized fat droplets which fill the cytoplasm of the cells, the Kupffer cells being not fluorescent. In salt water fish, the green fluorescence is of extreme strength and the liver structure can be made out only after the fluorescence has partly faded. In fat stains of livers from fresh water fish an identical picture was found. However, only a few of the fat droplets imparted the green, quickly fading fluorescence, whereas the majority show a yellow-brown, slowly fading fluorescence. This is due to vitamin A_2, a factor with an absorption spectrum

at 352 mμ, demonstrated by physico-chemical analysis in livers of fresh water fish in addition to small amounts of vitamin A_1 (114, 115, 116). Oils extracted from fresh water fish showed the yellow-brown fluorescence. If fed to vitamin A deficient rats, the yellow-brown fluorescence appears in the liver distributed in a manner similar to that of the usual green fluorescence and, as described (118 116, 117), growth of the rats continues. The different histologic distribution of vitamin A in the livers of fish supports the idea that vitamin A plays a special rôle in the metabolism of fish (33).

ADRENAL GLAND. In the cortex of the adrenal of the rat large amounts of vitamin A are present in small lipoid droplets in the epithelial cells of the fascicular layer. The glomerular layer, despite identical fat distribution, is free of vitamin A, even in hypervitaminosis in which the fluorescence extends almost to the medulla. In rats with low vitamin A reserves, the outer zone of the fascicular layer retains it (38).

In the human (26), vitamin A fluorescence is found in the fascicular layer of the cortex and to a minor degree in the glomerular layer, also in the central cortex close to the medulla. The fluorescence is less vivid and fades more slowly than that of the liver. Double refraction and vitamin A fluorescence were often concomitant and at times in identical distribution. The reticular layer reveals brown-red fluorescent particles (lipofuscin) which are also seen in the fascicular layer after fading of the vitamin A fluorescence. The variations of the vitamin A fluorescence are not necessarily parellel to that of the lipid content. Only small amounts or traces are observed in malnutrition and diseases of the liver, also in infectious diseases, here probably related to the depletion of the lipids (119, 120). The extensive changes of the human adrenal in early postnatal life (121, 122) influence the vitamin A distribution. During the entire first year only small amounts are found and then the storage gradually approaches that in the adult.

The adrenals are a storage place for various lipoids and one of the substances in the lipid mixture gives vitamin A fluorescence. Its slow fading may be caused by the presence of carotenoids which are responsible for the yellow color of the organ.

TESTICLES. In human testicles (26) after puberty, different fluorescent details appear which partly reveal a green, fading fluorescence suggestive of vitamin A: brown pigment in the cells of Leydig, and lipoid droplets and lipofuscin in the Sertoli and spermatogenic cells. The fluorescence of the Leydig cells is strong but slowly fading. The vitamin A fluorescence seems related to the activity of the testicle, appearing with maturity and disappearing with involution. In some animals the interstitial cells of Leydig show strong vitamin A fluorescence, whereas in others it is missing.

OVARY. In the rat ovary (38) a dull but slowly fading vitamin A fluorescence is seen in the interstitial cell cords and a bright and rapidly fading one in the so-called corpus luteum. In vitamin A deficient rats no vitamin A is seen in the ovary; upon feeding, it reappears.

In the human ovary the vitamin A fluorescence changes during development

and with the menstrual cycle, as studied on material obtained at autopsy (26) and from surgical operations upon women whose menstrual history could be ascertained (123). Three types of characteristic fluorescence are encountered: 1, a very dull, very slowly fading, diffuse, green fluorescence in the cytoplasm of the granulosa cells of the corpus luteum, probably due to carotene (carotene is found chemically in the ovary (124) and causes the yellow color of the corpus luteum); 2, a bright quickly fading fluorescence seen particularly in fine fat droplets in granulosa cells, and 3, a little duller, more slowly fading one in fat droplets in theca cells.

Vitamin A fluorescence appears around the third month of postnatal life, in fine fat droplets in the stroma, and in granulosa and theca cells of the Graafian and atretic follicles. In the reproductive stage the granulosa cells of the maturing follicle reveal fine fluorescent droplets. They increase in size and number after ovulation as the corpus luteum forms. The appearance of the granulosa cells in the mature corpus luteum suggests the possibility that the fluorescent droplets are secreted into the capillaries. With involution the picture of secretion changes into that of retention. The shrinking granulosa layer contains large, strongly fluorescent droplets and fine fluorescent droplets appear in the surrounding tissue. Finally, the vitamin A fluorescence disappears. The corpus luteum of pregnancy shows only carotene and no vitamin A fluorescence. The corpora atretica, which are rich in estrogen and progesterone (125), show strong vitamin A fluorescence of the double refractile lipids in the theca cells. In the course of the atresia it gradually disappears, while a red-brown ultraviolet stable fluorescence persists. The vitamin A fluorescence of the corpora atretica depends on their size and age and the menstrual cycle. The smaller and older the corpus atreticum, the lower the fluorescence. After the climacterium no vitamin A fluorescence is seen in the ovary.

The significance of the morphologic lipid distribution in the ovary and its relation to endocrine function is not established (126, 127, 128). The vitamin A fluorescence, however, seems to be a morphologic sign of endocrine activity. Whether it indicates hormone storage or formation is not clear. As long as the relation of vitamin A to sex hormone activity and even to reproduction is as unclear as at present (literature, see 26, 123), the presence of vitamin A fluorescence at the morphologic sites of sex hormone activity is best explained by solubility of sex hormones and vitamin A in the same lipid carriers.

Uterus. The endometrial lipids, present in certain stages (129, 130), occasionally reveal quickly fading vitamin A fluorescence without parallelism to that in the ovary (123).

Breast. The non-lactating mammary gland is free of vitamin A fluorescence. The fat droplets in the epithelial cells of the lactating gland reveal high amounts of it (14, 26) in agreement with the high vitamin A content of the colostrum (131).

Fat tissue. The fat cells in adipose tissue or scattered in the stroma of various organs impart a dim but slowly fading vitamin A fluorescence, in agreement with their function as vitamin A depots. The lipidic pigments retard the fading

and dim the fluorescence. The different depots of the same individual vary in their fluorescence and show some relation to the nutritional condition (26).

Eye. The presence of vitamin A in the eye is established (see 132). The vitamin A fluorescence, as reported by Querner (8) and Evans and Singer (133), changes depending on the function as described by Jancsó and Jancsó (13), Greenberg and Popper (134), and most carefully by Schairer and Patzelt (16). The pigment coat shows fine fat droplets arranged on the periphery of the epithelial cells. They reveal yellow, ultra-violet, stable fluorescence which may be covered by a green, fading vitamin A fluorescence. In the rod and cone layer, some diffuse vitamin A fluorescence is found in addition to a brown-red ultra-violet stable fluorescence which, according to Schairer and Patzelt (21), is due to visual purple. Vitamin A fluorescence is also imparted by connective tissue cells in the ciliary processes.

The nutritional status determines the vitamin A fluorescence of the ciliary processes just as it does that of other organs, but hardly influences that of the pigment layer. In the latter it is not increased in hypervitaminosis and not apparently decreased in hypovitaminosis. Even in far advanced vitamin A deficiency, long after the other organs have lost their vitamin A fluorescence, it is still observed in the retina and can be found even in the ulcerated eyes from extreme vitamin A deficiency. The state of light adaptation, however, which does not change the vitamin A in the ciliary processes, influences the fluorescence of the pigment layer and rod and cone layer. In dark-adapted animals no vitamin A fluorescence is seen, but the visual purple fluorescence of the rod and cone layer is strong. Upon light adaptation the latter disappears, whereas the vitamin A fluorescence is strong. The appearance of vitamin A upon exposure to light is rapid and takes place also in dead animals; its disappearance in the dark is slow. The fluorescence microscopic examination corroborates the hypothesis of Wald (135), that light changes the visual purple over a transitional stage of visual yellow and the carotenoid retinine to vitamin A and that during dark adaptation the visual purple is slowly rebuilt from vitamin A.

Intestine. The gastro-intestinal tract of the rat reveals vitamin A in its fat cells. Only after feeding of more than 900 units of vitamin does it appear in the wall of the duodenum and small intestine as a sign of its absorption. It is first seen in epithelial cells and in histiocytes and lacteals of the lamina propria of the villi, later on in larger lacteals up to the subserous layer (38). This picture of lymphatic absorption agrees with experiences in the human (136). Recent experiments (137) showed that vitamin A absorption after administration of oily concentrates starts in the upper duodenum, has its peak in the upper jejunum and subsides in the ileum. That does parallel the fat absorption. Whereas fat is found in the lumen throughout the entire intestine, its vitamin A fluorescence disappears above the level of the intestine at which the vitamin A absorption ceases, even after feeding of more than 300,000 units. The absorption subsides, therefore, either because vitamin A has been destroyed within the lumen of the intestine or because the entire vitamin A has been absorbed. In the human intestine no vitamin A fluorescence is encountered (26).

KIDNEY. In the kidney three types of distribution of vitamin A fluorescence occur in different animals. In the rat (38) a bright, quickly fading fluorescence is imparted by the endothelial cells of the intertubular capillaries of the cortex. It is marked in hypervitaminotic animals but also in advanced hypovitaminosis or in choline deficiency (62) when in both instances the liver is nearly depleted. Similarly the renal vitamin A concentration increases in depletion of the liver after dibenzanthracene administration (76).

In the normal dog kidney vitamin A fluorescence is imparted by medium sized isotropic fat droplets in the Henle loops.

In the human kidney (26) vitamin A fluorescence is only encountered in renal damage. The fluorescence is seen chiefly in the proximal convoluted tubules in fine droplets at the base of the epithelial cells. In the vicinity, the stromal histiocytes are rich in fluorescence. The vitamin A fluorescence is usually associated with marked fatty changes, but not necessarily with deposition of doubly refractile lipids.

The significance of the different forms of vitamin A fluorescence is unknown. In the human its appearance seems to be associated with the development of the nephrotic syndrome. Vitamin A appears in the urine together with other lipoids in renal diseases, but also in infections (see 138, 139). Together with other lipids and with protein, it seems to be reabsorbed in the tubules and carried from there to the tubular capillaries, sometimes being deposited in the interstitium (140). This explanation would agree with the recent theory on the histogenesis of lipoid nephrosis as a primary disease of the glomeruli with secondary tubular reabsorption of the lipoids and proteins (141, 142).

SKIN. Although the epidermis shows very early manifestations of vitamin A deficiency, no vitamin A fluorescence is encountered in the epithelial layers, even after feeding of high doses of vitamin A. Only the fat cells in the cutis and subcutis reveal small amounts (143).

OTHER ORGANS. In the rat (11, 38), after feeding high doses vitamin A may appear in the endothelial cells and connective tissue cells of lung, serous membrane, meninges and in other places. In the human, fluorescent fat droplets may be seen in the anterior lobe of the pituitary gland (8, 14).

TUMORS. Vitamin A fluorescence is seen in tumors originating from a mother tissue with vitamin A fluorescence, its distribution resembling that of the mother tissues (144). It may be found in hepato-cellular carcinoma, in adrenal cortical adenoma resembling the glomerular layer, in lipoma and in testicular dysgerminoma. Among the ovarian tumors it is chiefly present in those with endocrine activity. It occurs in the masculinizing arrhenoblastoma and in the feminizing group: granulosa cell tumor (in a distribution similar to that of the normal granulosa), theca cell tumor (resembling theca cells) and xantho-fibroma (simulating ovarian stroma with vitamin A fluorescence); while the ordinary fibroma of the ovary is without fluorescence. It was also observed in ovarian dysgerminoma (in a distribution similar to that of the testicle). The picture of a tubular adenoma of the breast resembled the lactating breast. Except for a few examples (*e. g.*, plant cells), the other tumors were free of vitamin A fluorescence,

even if they showed marked fatty degeneration. The presence and distribution of vitamin A fluorescence may help in determing the origin of a tumor. Thus the hypernephroid carcinoma of the kidney often reveals vitamin A fluorescence similar to that seen in the adrenal. This supports the original hypothesis of Grawitz, that those tumors originate from the adrenal (literature, see Ewing 145). Other renal tumors like carcinoma of the pelvis or benign tubular adenoma have no vitamin A fluorescence despite the presence of anisotropic lipids in the latter. That only a few well defined groups of tumors yield vitamin A fluorescence indicates that the local presence of vitamin A does not inhibit tumor growth, neither is it necessary for tumor formation. This is important in view of the repeatedly (see 144, 146, 147) discussed relationship between vitamin A and tumor formation.

Relation of vitamin A fluorescence to fat. The vitamin A fluorescence is, with few exeptions, bound to lipids demonstrable by sensitive strains. Vitamin A (or the other substances yielding the fluorescence) can be considered as exhibiting a fluorescent vital stain characterizing certain lipids of the body. However, since only some lipids reveal vitamin A fluorescence, the presence of vitamin A is not due to a non-specific solubility of vitamin A in the available fats but to a specific affinity of certain lipids for vitamin A (14, 148).

There is an apparent influence of vitamin A upon the fat metabolism in rats put on a vitamin A deficient diet. In agreement with chemical analyses (149), the morphological demonstrable fat disappears from the liver shortly after vitamin A vanishes; after feeding a fatty diet containing vitamin A, fat reappears one or two hours before vitamin A becomes visible (38). Various investigations (78, 76) indicate that vitamin A may occur also in a protein bound form; this is not excluded by the fluorescence microscopic picture.

The relation between the vitamin A depots in the body. In rats on vitamin A deficient diet the fluorescence disappears from different sites in the following order: interstitium of the various organs, adrenal, liver, and kidney; while the retina does not give it up at all. In repletion by oral route it reappears first in the intestine, then in the liver and almost simultaneously in the adrenal, finally in the kidney and the rest of the organs (38).

Upon carotene feeding, sometimes the vitamin A fluorescence appears first in the lamina propria of the villi of the small intestine but usually first in the Kupffer cells and shortly afterwards in fine droplets in the epithelial cells of the liver and in the adrenal (38). The question as to where carotene is split to vitamin A is therefore not yet satisfactorily answered. Most of the evidence points to the Kupffer cells of the liver (see also 100). However, sometimes cells of the intestinal villi or epithelial liver cells seem to split it.

Review of the vitamin A distribution in tissues. So far, not much can be concluded from the histological picture as to the rôle of vitamin A. Where the first morphologic signs of vitamin deficiency appear, namely, the epithelium (55), vitamin A fluorescence is not demonstrable. Actual presence of vitamin A is required for its rôle in vision; it participates in a known chemical process in the retina from which it does not disappear even in extreme deficiency. The

different localizations of vitamin A may be due to the following factors: 1, participation in a chemical process (retina); 2, distribution and possible destruction (Kupffer cells); 3, presence at the site of sterone hormone formation (adrenal, testicle and ovary), probably due to solubility in the lipid carriers of the hormones; 4, storage (fat cells and cells of the interstitial tissue in the rat); 5, absorption (intestine); 6, secretion (lactating breast); 7, pathologic permeability (kidney); 8, imitation of the mother tissue (tumors). Its presence in the epithelial cells of the liver is due to several of the above factors.

CONCLUSION

The microscopic visualization of vitamin A by fluorescence permits the study of its distribution in organs. In various physiologic and pathologic conditions the distribution undergoes characteristic changes, not indicated by chemical analysis or bio-assay. Conclusions as to the rôle of vitamin A in the body can be made which are not arrived at by other methods. The histologic method, which permits only a rough but quick estimation as to the quantity of vitamin A present, does not substitute for the chemical analysis but supplements it. The fluorescence microscopic demonstration of vitamin A, however, not only aids in the investigation of the vitamin A metabolism but also presents a new histologic method for the differentiation of lipids independent of the biologic significance of the fluorescence.

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THE HISTOPATHOLOGY OF RADIATION LESIONS[1]

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The histopathology of lesions induced by radiation is the resultant of several diverse changes. Some changes are cellular, as necrosis of cells irradiated, alteration of cellular function, or chromosomal changes induced in cells which lead to death or alteration of function of cells subsequently developed from them. Some are intercellular (probably in part mediated through injury to the cells maintaining such substances as collagen, osteoid, or elastica), varying from slight alteration in texture to necrosis. Some are vascular. Since endothelial cells and other components of vascular walls are fairly sensitive to radiation, vascular changes ranging from thrombosis to endothelial swelling as early manifestations and from telangiectasia to complete or partial occlusion as late alterations may be induced. These altered vessels, often inconspicuous in themselves, may bring about profound changes in those tissues dependent on such vessels for their blood supply.

The character of the pathologic alterations produced in any tissue depends on its cellular pattern and the degree and extent of exposure to radiation. Radiation lesions vary markedly according to whether they are recent or long standing and according to the tissue in which they occur. All, however, have certain features in common and it will be the function of this review to consider these particularly.

The types of radiation producing the lesions which will be discussed here are those used therapeutically: electro-magnectic radiation of a wave length ranging from 10 to 0.05 Ångström units, corpuscular radiation as the alpha and beta radiation from radium or certain of the temporarily radioactive isotopes, and neutrons. So far as one can determine, the fundamental biologic effects of these different agents are the same and are dependent on the degree of ionization produced by them in the tissues and the localization of the ionization within the cells.

A fascinating recent development in the field of therapeutic radiation has been the utilization of temporarily radioactive isotopes (1, 2, 3, 4). The peculiarities of certain of the body's cells in utilizing one or another chemical substance can serve to concentrate radiation effect in those particular regions if the proper isotope is selected. Thus Marshak (5) has shown that radioactive phosphorus is taken up by the nuclei of rapidly proliferating cells ten times as rapidly as it is by the nuclei of resting cells and by utilizing radioactive phosphorus one therefore can get a consolidation of radiation effect on those tissues

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whose cells are undergoing rapid proliferation. Striking therapeutic results have been obtained in leukemia and polycythemia. Similarly radio-active strontium is segregated by osteoblasts and laid down to a considerable degree in bone, thus concentrating radiation in regions of osteoblastic activity. Unfortunately, in the case of tumors of the thyroid, the neoplastic cells rarely show the avidity for iodine that normal thyroid cells do and hence the hoped-for therapeutic effect from the use of radioactive iodine in thyroid tumors could not be obtained.

It must be remembered that so far as immediate radiation effect alone is concerned, tremendous doses are required to kill all of a given group of cells, even though slight doses will kill some. Radiation does not affect equally all cells within the field, even at an equal depth beneath the skin and an equal distance from the source, because of the discontinuous character of the energy applied. As an example of this character, we may quote from Crowther, "Suppose that it were possible to pass, through the air of an ionization chamber, X-radiation at an intensity of 1 r per second continuously, day and night, for 500 years, we should still leave about one third of the molecules unirradiated" (6).

In any consideration of the dosage of radiation, it is important to know the physical factors of that radiation. The effect is proportionate to the amount that is absorbed, rather than the amount as measured by the ionization chamber. The "harder" the radiation (the shorter the wave length), the greater will be the penetration of the tissues and the less will be the damage to the skin by a given dose. The "hardness" increases with voltage and with filtration. "Cross-firing" through different skin portals toward a tumor will sometimes permit giving a greater depth dose to it than any one area of the skin receives. The effect is also influenced greatly by the size of the field. This is due in part to the fact that the "scatter", or the number of electrons separated from the atoms irradiated and giving secondary radiation, increases for a given dose of radiation with the increase in mass exposed. Thus, because of this secondary radiation, the total amount of radiation per given dose delivered will be much greater per cubic centimeter of tissue if the field of radiation is 10 x 10 cm., than it is if it is 3 x 5 cm. Irradiation of the entire body is very potent in man; a dose of about 500 r kills. The rate of delivery of radiation is also important; for example a dose of 3000 r delivered in two hours is far more destructive than the same total dose fractionally delivered at daily intervals for 10 days. One-tenth r per day is the accepted tolerance dose for man, but may be a trifle high (7).

Not too successful attempts have been made to establish biologically equivalent doses of radiation. However, one can estimate to a limited degree the amount of beta radiation, of neutrons, of x-rays required to obtain similar degrees of injurious effect on tissues.

Alpha particles, although virtually devoid of penetrating power, are about one hundred times as effective in injuring cells as beta rays. Both are less penetrating and more destructive than gamma rays, which are equivalent to the hard roentgen-rays produced by supervoltage apparatus. Accepting skin erythema as an index, well-filtered radiation at 1,000 KV requires $\pm$1200 r;

at 200 KV, 700 r; and unfiltered radiation at 100 KV, 320 r. Neutrons are apparently from one to fifteen times as potent biologically as physically equivalent amounts of roentgen rays.

The r, the unit of x-ray radiation, is merely a measure of the ionization of air by roentgen rays under standard conditions and a dose expressed in r alone gives little information as to the biological effects that may be expected.

A word should also be said with regard to the measurement of radiation given off by temporarily radioactive isotopes. Their amount of radioactivity is commonly expressed in millicurie equivalents, that is, the effect that they produce on a standard ionization chamber as compared with radiation from 1 mgm. of radium. This is a physical measurement and does not imply any direct biological comparability of effects with a similar dose of gamma radon radiation measured in millicurie hours.

In spite of the different types of radiation, the different wave lengths and the different intensities of radiation experienced, the pattern of response of the tissues, once the threshold of reaction of their components has been reached, varies quantitatively rather than qualitatively (8).

The assumption that small doses of x-ray or radium radiation are stimulating (the Arndt-Schulze "law") is invalid. The slight evidences of proliferative activity offered as evidence by the proponents of this hypothesis are in fact only reparative responses to the injury that has been done. On examination of a wide range of tissues irradiated to varying degrees, I have found no evidence of a stimulating effect.

Different tissues do not show unique changes. Certain combinations of changes are characteristic (9) as will be pointed out later. Thus, though radiation tends to produce abnormal mitoses, vascular injury, and hyalinization of collagen, these may all be produced by other substances; as lipoid soluble anesthetics, in the case of multiple mitoses; the heat of cauterization, in the case of hyalinization of collagen; and a wide variety of physical and chemical agents in the case of vascular damage. The early responses to radiation injury seem to be in part concerned with altered fluid balance: edema of the tissues and swelling and vacuolization (10) of the cells are prominent features.

The histopathologist can say with a fair degree of accuracy that a given tissue effect is produced by radiant energy, but he cannot so state with infallibility.

The combination of two or more of the non-specific characteristics of radiation is strongly presumptive evidence that the injury is in fact the result of radiation. Thus the combination of giant and irregular nuclei, hyaline connective tissue, and thick-walled hyalinized blood vessels would be difficult to explain on any other basis than that of a late response to irradiation.

It was noted early that various types of cells and various tissues show injury after widely different doses of radiation. Viruses may be unchanged by radiation of several hundred thousand r. Some fibroblasts in vitro may survive 100,000 r, whereas frog sperm may be profoundly altered by as little as 25 r. This variation of response of different cells and tissues is of great importance clinically. It is this variation in response which permits radiation to be used

as a therapeutic agent producing injury selective, at least to a degree, for certain abnormal cells or tissues while sparing others.

In general, one classifies tissues or tumors as radiosensitive if there is gross change with radiation of less than 2500 r given under ordinary therapeutic conditions. Such radiation does not usually injure most tissues permanently. A tissue is considered radioresponsive if such alteration occurs with a range between 2500 and 5000 r and radioresistant if over 5000 r is required for a response. At or above this dose some permanent injury is done to a number of normal tissues (9).

That variations in sensitivity to radiation are not necessarily dependent on specificity of protoplasm is shown by the extraordinary variations in effect on Drosophila at different stages of the life cycle. The ova have a median lethal dose of 155 r and the late pupae have a median lethal dose of 95,000 r. Fortunately for tumor therapy, cells in mitosis are much more sensitive than resting cells. Other physical agents, of course, vary in the extent of injury they cause to different stages of development of the same organisms, but not to so great a degree. Thus eggs of *Ankylostoma caninum* are readily killed by freezing with dry ice, whereas the larvae live frozen for weeks (11).

The assumption has been made (the law of Bergonié and Tribondeau) (12) that more primitive cells are more sensitive to radiation. For example, the lymphocyte is the most easily injured of somatic cells and the neuron is one of the most resistant cells of the body as might be expected from its high degree of organization and its low metabolic activity. While this assumption holds to some degree in the animal organism, it is not a consistent rule. Thus the relatively simple organism *Chaos chaos*, or giant ameba, is extraordinarily resistant to radiation while the specialized ciliated epithelium of the mammalian bronchus is highly sensitive.

Much of our knowledge of variations in response of different cells, tissues, and tumors is empirical. Several valuable reviews of radiation effects in general are available (9, 13, 14, 15, 16, 17, 18) as well as of effects on tumors (19).

Injury, whether to tumor tissue or normal tissue, due to therapeutic irradiation, unlike that due to other physical agents, is not immediately apparent on inspection at the site of the injury or elsewhere. Several days may elapse between the time of irradiation and the first visible changes in the skin. With ordinary therapeutic doses, this latent period in all probability is only apparent, as intracellular changes have been shown to develop almost immediately following the onset of irradiation. The height of reaction may not be reached for several weeks and if the dose has been heavy, new evidence of injury may develop even after months or years. Much of our present interpretation of the so-called latent period rests on experiments performed in the early days of radiation and carried out with variable physical factors owing to both mechanical inadequacy of the source of radiation and inadequate physical means of measuring the amount of radiation given. It might well be profitable to reinvestigate some of our knowledge of the latent period.

In some cells and particularly in the germ cells, the latency in appearance

of injury is real. Only a portion of a single chromosome may be damaged and no effect may appear until a number of mitoses have taken place, finally resulting in the development of a cell in which the original masked chromosomal injury will become apparent through marked alteration in form, function, or even in cell death (20).

One common assumption has been made by both laymen and physicians, that in the course of treatment of a given lesion by radiation any permanent damage to normal tissue or the production of an x-ray "burn" represents evidence of incompetence. This is not true. Radiation cannot be given effectively to any tissue without at least some effect on adjacent tissue and the character of that effect hinges not only on the skill of the radiologist but on the dosage of radiation which it was necessary to deliver to accomplish the therapeutic end desired. As more and more patients are cured of tumor by radiation, we will inevitably see undesired late sequelae of radiation, varying from atrophic skin and disfiguring telangiectasis, to actual extensive necrosis.

The sensitivity of cells varies not only between types of cells themselves but even in the same cell with various stages in its life cycle. Thus the nuclear chromatin in general is affected more than is the cytoplasm, and due to its relative importance, nuclear injury has profound effects on the cell as a whole. The nucleus in mitosis is particularly sensitive. In the human and many animals, the early prophase is apparently the stage in which the cell is most susceptible to radiation injury. With a fairly intense source of radiation, there will be decrease in mitotic activity within one half hour of the onset of radiation and there will be practically complete cessation of mitotic activity up to twelve hours with recovery under way by eighteen hours, provided the cell survives (21). While injury to nuclear structures may be done at all stages of mitosis, by and large mitosis once initiated tends to continue through to completion. Evidence of radiation injury may be apparent in these continuing mitoses, however.

The types of visible change produced are 1, inhibition of mitoses; 2, breakage of chromosomes; 3, lagging of chromosomes; 4, complete or partial adhesion of chromosomal pairs; 5, asymmetry of mitosis. There is a marked tendency for multipolar mitoses to develop following radiation (22). Three stages of mitotic injury were noted by Politzer (23): the immediate stage with inhibition of mitoses, pseudo-amitosis and pyknosis; the intermediate stage, during which mitotic activity is practically absent; and the later stage during which abnormal or multiple nuclei are formed as a result of a division of nuclei whose chromosomes have been injured. This period of abnormal mitosis is most marked from 24 to 96 hours after irradiation in case of some tumor tissues. The occurrence of multipolar mitoses is, of course, accompanied by and perhaps dependent upon the occurrence of multiple centrioles and as many as 50 per cent to 80 per cent of interkinetic cells may present multiple centrioles following radiation (22). It is important to remember that the visible injurious effects on the nucleus and on mitosis are not pathognomonic of radiation and may be produced by heat, ether and other means.

The effects on the cytoplasm and its constituents are less well understood than

those on the nucleus, in part because it is difficult to tell what cytoplasmic alterations are primarily due to nuclear injury and what are actually direct effects on the cytoplasm itself.

The first cytoplasmic change noted in tissue culture is the cessation of motility and the retraction of pseudopods with a resultant rounding up of the cells.

Perhaps the most consistent feature noted in the cytoplasm is vacuolization (10). This occurs in a high proportion of cells damaged by radiation and is most apparent in those going on to necrosis. This vacuolization suggests an altered permeability in the cell membrane. The vacuolization is due at least in part to inhibition of fluid as the cells increase materially in size, roughly paralleling the degree of the vacuolization. Other constituents of the cytoplasm are not particularly involved. The mitochondria will be injured by heavy radiation but this injury is not characteristic. The Golgi apparatus is sometimes fragmented and appears swollen with a sublethal dose of radiation. Recovery usually occurs.

In organized tissue preparations, loss of cell function is early apparent. Thus the cilia of respiratory epithelium cease to function very promptly after the onset of radiation. The power of phagocytosis is lost within a few hours and the secretion of mucus in the intestinal tract is increased very strikingly within some days. The secretion of acid by the gastric mucosa may be brought about a few days after radiation and will persist for some two to four months. Dryness of the mouth due to deficient salivation is a frequent accompaniment of therapeutic radiation about the mouth.

Among the interesting effects produced by radiation on intracellular tissues are the changes in bone (24). The characteristic feature is the maintenance of integrity of structure with eburnation and devitalization without leukocytic reaction. The osteoblasts disappear, the osteocytes are absent and the lacunae which they occupied are apparent as empty spaces. Frequently the canaliculi are enlarged and very irregular but reduced in numbers. Abnormal spaces may appear about the lamellae. As a rule the density is markedly increased and an almost ivory-like texture is apparent. This is emphasized in the x-ray, showing as dense zones. This maintenance of structure in spite of heavy radiation is due in part to the slowness of metabolic processes in bone and in part to the existence of its amorphous substrate.

Osteoclasts are apparently slightly more sensitive than are osteoblasts and at least a portion of this eburnation may result from a reduction in the usual resorption of the bone. More rarely injury will be apparent as rarefaction as a result of excessive resorption and in these instances it is due to halisteresis rather than to osteoclastic over-activity. The changes in the osteocytes themselves are not striking; pyknosis, vacuolization and disappearance following more or less in sequence. A striking feature is the absence of any zone of demarcation between the normal and irradiated bone, one shading off into the other. There is no sequestration in the ordinary sense. Usually when sequestration occurs, it is because of the advent of secondary infection. Such infection may develop in irradiated bone, and when it appears the picture resembles

chronic osteomyelitis of long standing. Irradiated bone is more susceptible to infection than is normal bone because of its poor circulation, low cellularity and necrosis. Necrotic bone acts as an infected foreign body.

The cells of the epiphyseal line are much more easily injured than are osteocytes. The columnar pattern becomes disorganized and some cells are damaged and disappear (25). If the dose of radiation has been slight, regeneration occurs, but if heavy, growth at the epiphysis will be lessened or cease. However, a moderate dose of radiation will not hamper healing of a fracture.

In considering the result of irradiation of cells, their relative sensitivity must be kept in mind. If somatic cells were to be arranged in order of decreasing sensitivity to radiation, there would be first lymphocytes, then granulocytes, then epithelium, smooth muscle, fibroblasts and their derivatives, and finally neurons. The sensitivity of the germ cells is probably of the same order as that of the lymphocytes. The immature ova are apparently more resistant than are the matured ones, since a dose of 1200 r at 200 KV potential, for example, administered to the ovaries of a woman in the childbearing period will induce amenorrhea and sterility, usually followed in a number of months by a resumption of the menstrual cycle and fertility.

The effect of radiation on germinal cells must be considered as somewhat different from that on somatic cells. While x-ray injuries to normal tissues were noted within a few months after the discovery of roentgen radiation, the sensitivity of the ovary and testis was not noted until eight years had passed by. Bergonié and Tribondeau (12) pointed out that the more primitive cells, among which are the germinal cells, were more susceptible to radiation than better differentiated cells. In recent years, chiefly through the work of Muller (20), the effect of radiation on germ plasm received added emphasis through its application to genetic studies. The initial technique was simple. Male Drosophila were irradiated and mated with normal virgin females. The offspring were then examined for abnormalities. In general, the normal mutation rate is practically doubled in Drosophila by a dose of 30 to 40 r applied to the germ cells lying in the gonads. Viable mutations occurred only one-tenth as frequently as lethal mutations. Further evidence has been brought out by the mutation of chromosomal aberrations (26).

Henshaw (7) concludes that x-ray-induced mutations are a result of local rather than general exposure, that the results of previous exposures are additive, that the duration and frequency of exposures do not influence the reaction, that recovery does not occur, and that general idiosyncrasy is important.

It may be fair to assume that irradiation of human gonads would give the same proportion of lethal mutations to viable mutations as in Drosophila, namely, ten to one. This might account for the relatively low incidence of abnormal embryos in the human. The experimental data have been reviewed by Murphy (27) and human data have been presented by Jost (28) and by Hickey and Hall (29). In Jost's series of 63 pregnancies following pelvic irradiation of 141 women, there were 14 abortions and 41 live children; only one of these was abnormal, showing underdevelopment with strabismus and myopia. Hickey

and Hall (29) obtained information from 377 radiologists with regard to fertility and abnormalities of offspring. Thirty-seven per cent of the couples had no children, and of the 412 children 4 per cent showed abnormalities.

There is no question but that moderate to heavy radiation of the embryo is deleterious. If the irradiated embryo survives, the abnormalities that subsequently develop may be quite characteristic. The eyes are frequently involved, the epiphyseal lines may show evidence of injury with striking alteration in the growth of the bones involved (usually those of the extremities). Goldstein and Murphy (30) reported on 75 children who had been irradiated in utero. Thirty-eight showed defects or serious ill health, 20 of the 75 showed central nervous system changes and 16 per cent of the group were microcephalic.

This distinction between somatic cells and germ cells has an important relationship to the whole problem of radiation effect, because the protection planned to avoid somatic tissue injury may not be adequate for protection of the germ plasm. The ordinarily accepted tolerance level for protection is 0.1 r per day. In establishing any level for the tolerance dose, it is important to remember that other criteria exist than the obvious ones often adopted, the development of dermatitis or sterility. The lymphocytes have long been established as the most sensitive of the somatic cells. As has been noted by Russ (31), Henshaw (7), Warren (32) and others, the lymphocyte count in the peripheral blood is probably the most sensitive index that we have of somatic radiation injury. This is manifest by a lowering of the lymphocyte count followed by recovery in 12 to 20 days, frequently still later followed by an over-compensation with a lymphocytosis of fairly long duration.

In most somatic cells, 3 different stages of injury may be recognized as of increasing severity: alteration of function, alteration of structure, and necrosis. Not all stages occur in all cells irradiated, and rarely the descendants of surviving cells may become neoplastic.

The rôle of infection in determining the response to radiation either in non-neoplastic or neoplastic tissues is important, but little understood. Clinical experience has demonstrated that infected tumors are more resistant to radiation than are the same tumors free from infection. The mechanism involved is unknown. That irradiation of slight intensity is of value in the treatment of certain infections has been proved, particularly in the case of acute parotitis. Here the hyperemia induced is probably of importance.

Tissues damaged by radiation are susceptible to infection. Infection, if it occurs, accentuates particularly the late deleterious changes. In interpreting the course of infection in tissues that have been irradiated, two factors must be kept in mind: first, the impairment of the vascular supply greatly hampers the normal defense mechanisms of the body; second, the dense hyalinized intercellular substance of irradiated tissue supported by cells already injured, when exposed to infection is readily killed and acts as a sequestrum keeping alive the infection. An excellent example of this type of change may be seen when the presence of a carcinoma of the gum has made necessary heavy radiation of the region of the jaw. Usually, in order to eradicate the tumor, it is necessary to

use a sufficiently great dosage to cause marked injury to the surrounding normal tissues. Through the ulceration opened up by the necrosis of the tumor, bacteria from the mouth may gain access to radiation-damaged bone and to hyalinized collagen of the fascia. Since in irradiated bone there is no clear cut transition from healthy bone to that injured but rather a gradual transition from healthy bone through that slightly damaged to dead, there is usually no sequestration. However, as the infection progresses through tissues with impaired blood supply and encounters already injured cells, it is not walled off and gradual disintegration of the more severely damaged bone occurs. This is usually discharged in fragments, followed by further necrosis induced by progress of the infection until ultimately the chronic osteomyelitis may extend well beyond the fields of radiation. Similarly, if the ulceration and infection extend to the fascial planes of the cheek, there will be progress of low grade infection along the relatively inert collagen of the fascia, many of whose supporting fibrocytes have been either killed or heavily damaged by the radiation. The fascia becomes necrotic, shreds, and is discharged in sequestrated fragments. Such a process of necrosis and sequestration may continue for several years.

Sometimes the effects of infection will be combined with those of trauma in producing lesions of clinical importance in irradiated tissue. As an example of this, we may consider the radiation ulcers of the intestinal tract (33). In the course of the irradiation of tumors of the abdomen and particularly those of the pelvis, it is inevitable that more or less exposure of the intestinal tract occurs, resulting in varying degrees of radiation injury. In observation of the radiation ulcers, one is impressed by the fact that they are much more localized than is the field of radiation and the resultant abnormality of the intestinal wall, and their localization is such that it is difficult to interpret them as occurring as a result of radiation alone. If we follow through the sequence of events in the lesions noted clinically and in those produced experimentally, it becomes apparent that ulceration occurs because of trauma and infection impinging upon an altered mucous membrane and bowel wall, structurally and functionally damaged by radiation and imperfectly regenerated. While it has been suggested that radiation ulcers might be due to obliteration of blood vessels as a result of the radiation, our observations do not confirm this.

Another example of the aggravation of radiation injury by trauma with or without infection is demonstrated in the case of chronic radiation injury of the hand. This lesion, exemplified well in many of the early x-ray workers, has been long studied and is as well understood as is any radiation lesion. Owing to the radiation changes of the epidermis, the cells are thin, abnormal, disarranged and hyperkeratotic. The cutaneous glands are absent, so that their secretions which aid in keeping the normal epidermis soft and pliable are of no avail in protecting the thin, scaly, irradiated epidermis. Moreover, as a result of the radiation injury, the rete pegs are largely smoothed out and the basement membrane is degenerated. The epithelium rests almost without structural bonding on the hyalinized corium, which is a poor substrate for even normal epithelium. The impaired blood supply further contributes to the lowered resistance to

injury and inability to repair, so that traumatism of the skin readily results in ulceration. Ulcers, once formed, tend to persist almost indefinitely owing to unfavorable conditions both for epidermization and combating of infection. As a result of abortive efforts of repair, often continued for years, the epithelium at the margin of these radiation ulcers may become carcinomatous.

Radiation injury of the skin has long been recognized, both because of the number of cases of radiation dermatitis or radiation carcinoma that developed in radiologists and in their patients and, second, because of the utility of the cutaneous erythema as a means of gauging radiation dosage. Therefore, at the risk of repetition, the histologic sequences will be considered. The gradual transition over a period of years from healthy, elastic, normal skin to dry, atrophic, smooth or focally hyperkeratotic skin may be followed with ease. The first change noticeable, immediately after irradiation, is a cessation of mitotic activity in the epithelial cells, followed in a few days by edema of the subcutaneous tissue with basal dilatation and evidence of vascular damage ranging from none to complete thrombosis. At this time the gross erythema and edema of the acute reaction are apparent. The collagen fibers, at first merely separated by the edema, become swollen and tend to coalesce. Later there is increased production of collagen with the formation of dense, almost glossy, hyaline intercellular substance. The elastic fibers swell, fray, and, depending upon the severity of the irradiation, either become increased in amount or disappear. With progress of time, the epithelium becomes thin and the rete pegs tend to disappear, leaving an almost straight boundary between the epithelium and corium, with loss of the basement membrane. This change, if it involves the fingers, may result in loss of the individually characteristic fingerprints. The skin appendages atrophy and disappear. There is an increased production of melanin by the basal cells. Distortion of the arrangement of the epithelial layers is followed by hyperkeratosis, sometimes focal in character, and the appearance of abnormal epithelial cells, sometimes with atypical mitoses. Such of the more superficial vessels and capillaries as survive are telangiectatic. The deeper vessels are usually partially, or some even completely, occluded, with some endothelial proliferation, by hyaline thickening of their walls. The epithelium, as it proliferates focally, penetrates somewhat into the corium, and finally becomes actively invasive (34). Years are required for the evolution of a carcinoma of the skin secondary to radiation. The observations of Wolbach (35) in this field are classical and deserve study by all those interested.

Radiation injury of several tissues as well as the skin, particularly that produced by repeated even though small doses, may lead to the development of neoplasms. The pattern of evolution followed is similar to that of many of the induced tumors: injury necessitating long continued reparative activity; abnormal environment of the proliferating cells and probably mutations induced in those cells. In the human I have seen epidermoid carcinoma, basal cell carcinoma, fibrosarcoma, osteogenic sarcoma, and myelogenous leukemia, which apparently developed on the basis of radiation injury. Also ascribed to the effect of radiation have been carcinoma of the lung (as seen in the Schneeberg miners and some radium workers), lymphatic leukemia and other lesions.

Since the greatest use of therapeutic radiation is in treating tumors, separate descriptions will be given of the radiation changes in examples of the radiosensitive, the radio-responsive, and the radio-resistant tumor.

As an example of histopathologic changes induced by irradiation of a radiosensitive tumor, we may select as a fairly typical example the response of a lymph node involved by lymphosarcoma. Within a few hours after the period of radiation there will be swelling of the node, which gradually goes down and at the end of several days the node, assuming the response has been satisfactory, will be barely palpable. If we follow microscopically through the course of events that underlies this series of changes, we will see first a cessation of mitotic activity in the tumor cells, with necrosis of cells, both those undergoing mitosis and cells in the resting stage. This necrosis is followed by autolysis of most of the necrotic cells, although some phagocytosis by macrophages of cells and cell fragments occurs. The increase in size noted grossly in the nodes during this period is the result of edema, probably the result of the autolytic process. This results after a few days in the histologic picture of the node being that of the reticular net and sinusoids distended with edema fluid, some phagocytic macrophages, and almost a complete loss of the lymphoid elements. However, a few persist and usually after some weeks or months the tumor process has reestablished itself and may be even larger than it was at the time of treatment. Owing to the light dose capable of accomplishing this change, very little, if any, change will be noticed in the supporting tissues. Usually the recurrent tumor (cure is very rare) is more resistant to irradiation than was the primary tumor.

As an example of the effects in a radio-responsive tumor we may take epidermoid carcinoma, grade III, of the cervix uteri. The effects of equivalent doses of radium and of x-ray irradiation, both of which may be used in the therapy of this tumor, are essentially similar. Very shortly after exposure mitoses cease, sometimes within an hour. Those cells in which the mitotic process has been initiated tend to carry through to completion but many of the cells die first. Since the radiation given therapeutically is well over the threshold dose, injury is done to the resting cells as well as to the dividing cells. The nuclei gradually become hyperchromatic and enlarge, chromatin tends to be clumped rather than dispersed, and the nucleoli become strikingly prominent. Calcification of the nucleus has been reported. The cytoplasm becomes vacuolated and the cells increase considerably in size. After 18 to 24 hours mitosis is resumed to some degree but the proportion of abnormal mitoses with unequal spindles, lagging chromosomes, chromosomal attachments or multi-polar spindles is appreciably higher than was previously the case. As a result of the multipolar mitoses, tumor giant cells with bizarre and giant nuclei may be formed. Other of the abnormal mitoses lead to death of the cell. Necrosis develops both as a direct result of irradiation and as a process secondary to vascular and stromal damage. Secondary infection may also play a part. The cytoplasm of the surviving cells tends to become more and more keratinized and sometimes the only residual cells will be giant forms with prominent keratinized cell membranes, intercellular bridges and kerato-hyaline granules. While these changes are taking place in the tumor cells, the stroma does not remain unaltered. The

fibrocytes of the stroma at first show no change but later some die and others become large, stellate rather than elongate, often vacuolated and may have irregular giant nuclei. The collagen tends to lose its fibrillar structure, the fibers swell and coalesce to form a mass of dense hyalinized material. Here and there in the collagen, foci of necrosis develop with deposition of fibrin. The elastic fibers swell at first and later partially disintegrate. The usual radiation scar contains but little elastic tissue capable of function.

Very early in the course of radiation there is swelling or even necrosis of endothelial cells of blood vessels. Where necrosis occurs and the vessel is small, there will be an occluding thrombus; if large, there may be only a mural thrombus. The smooth muscle cells of the media are swollen and necrosis of them may occur. Several weeks after irradiation the late vascular changes will usually have developed. There will have been either thrombosis and organization, or proliferation of the swollen endothelial cells to a sufficient degree to cause at least partial occlusion. In the smaller superficial vessels and capillaries, the damage to the wall and back pressure from narrowed veins may bring about a marked degree of telangiectasis. In the larger vessels, a marked narrowing or occlusion of the lumen results, due in part to endothelial thickening and proliferation and in part to thickening and hyalinization of the connective tissue and degeneration of the elastica and muscle cells of the media.

The initial tissue destruction by the tumor, the death of the tumor, the dense hyaline character of the connective tissue and the impaired blood supply lead to ulceration of the cervix which presents quite a characteristic appearance. Superficially there is a zone of relatively cell-free fibrillar necrosis rather reminiscent of that seen at the base of a chronic peptic ulcer. This fades off over a distance of 20 to 50 micra, passing through a stage of coarsely fibrillar, partly hyalinzed connective tissue to a zone of marked hyalinization. Here and there a few leukocytes may be scattered but in general their response is minimal. More superficially there may be telangiectatic capillaries and venules and thick-walled or completely occluded arterioles. As one progresses farther from the surface, there may be a few scattered tumor cells embedded in the hyaline tissue. These sometimes cluster into small islands. If the radiation has been sufficiently heavy, the tumor cells will not be present. If a recurrence of the tumor develops, the tumor cells as a rule are smaller, less keratinized and much more closely packed than they were during the period of response to radiation.

As an example of radio-resistant tumors, we may take an adenocarcinoma of the rectum. Here as a rule very heavy radiation has to be given to produce any significant effect. Most of the tumor cells will remain undamaged, some of them even undergoing mitoses in the face of a dose of radiation that will cause marked degenerative changes in the smooth muscle, connective tissue and blood vessels of the intestinal wall. Sometimes increased mucous secretion by the tumor cells results. Partial regression of the tumor may occur, chiefly through injury to its blood supply.

It is important not to confuse radio-sensitivity of a tumor with radio-curability of that tumor. Thus a lymphosarcoma is highly radio-sensitive but rarely

cured, while epidermoid carcinoma grade I of the lip is resistant but often cured if the dose is sufficient. Many of the tumors that respond well to radiation initially will recur, and usually in a form more resistant to radiation than were the cells of the original tumor. Moreover, the tumor may have metastasized prior to treatment and radiation does not affect metastases that may have developed outside the irradiated field any more than surgical removal would affect metastatic growths outside the operative field.

While in general the radio-sensitivity of tumors tends to follow that of the type cell from which they are derived, none the less there are striking variations from this and our knowledge of the radiation responses of tumors of various types rests on clinical observation rather than on *a priori* reasoning based on assumed analogous behavior of the corresponding normal tissues when irradiated. Ewing's tumor of bone is highly sensitive to radiation but osteogenic sarcoma of the bone is highly resistant. Leiomyoma of the uterus usually responds well to radiation, while leiomyoma of the intestinal tract does not respond so satisfactorily. Neuroblastomas respond well to radiation, while gliomas and melanomas are very resistant.

There are also variations in sensitivity determined by the stroma of the tumors. A relatively acellular supporting tissue, as cartilage or bone, gives a less favorable therapeutic reaction than does a cellular one, as new-formed connective tissue or invaded muscle. For example, basal cell carcinoma of the skin is easily cured by radiation when located on the cheek whereas one occurring on the forehead invading the frontal bone is extremely resistant. In general it can be said that increased amounts of intercellular substance in the supporting tissue of the tumor or secondary infection tend to increase resistance whereas a greater degeee of vascularity tends to decrease resistance.

Although the elements of the histopathologic response to irradiation are fairly simple, so many variables may operate to emphasize one and mask another that the lesions may vary widely and be difficult to diagnose until analyzed. With further study many of the responses of normal tissues and tumors should become more accurately predictable.

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THE BREAD PROBLEM IN WAR AND IN PEACE

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Bread, in the Bible, is a term used to signify food. Thus in Gen. III, 19, the Lord says to Adam "In the sweat of thy brow shalt thou eat bread." It is also expressed in the prayer "Give us this day our daily bread." Bread (whole wheat) is practically a complete food upon which populations have often leaned heavily for their source of nutriment and, indeed, it has well earned its age-old title, "the staff of life."

The separation of the ground whole wheat meal into the coarse bran and fine flour began very early, and the fine flour was often considered the choicest part of the wheat: "The Bible speaks of fine flour or meal, as a portion of the meat offerings to the temple" (1). The Romans had four or five grades of flour; the finest, from which all the bran was removed, was eaten only by the rich (1).

The ancients realized that wheat flour suffered in nutritive value when the coarse bran was removed, and their wrestlers "ate only the coarse wheaten bread to preserve them in their strength of limbs" (1). Hippocrates, the father of medicine, who flourished over two thousand years ago, recommended the unbolted wheat meal bread "for its salutary effects on the bowel" (1). Experimental evidence of the superior nutritive value of whole wheat flour as compared to white flour was furnished by Magendie, who might well be called the father of modern nutrition. He found that dogs fed on the dark coarse bread lived for a long time in good health, but declined and died in less than two months if the bread fed was made of high grade white wheat flour (2).

The "fine flour" referred to was not impoverished to the extent of modern white flours. Stone-milling ground the wheat germ and much of the bran so finely that they could not be separated out by bolting. The "fine flour" was not white but had a creamy color. Modern roller-milling operations do not grind the wheat germ but flatten it so that it can be bolted out of the fine flour. The bran is flaked and more completely removed. The small amount of carotene in the flour is destroyed by special bleaching processes. The bleaching process gives "the artist's touch" to man's ignorant abuse of his "staff of life." The increased efficiency of modern milling methods in destroying the nutritive value of "the staff of life" is shown in table 1 (2).

This efficient destruction of the nutritive value of whole wheat flour was perfected and came into general use after 1880 (2). It might well have had a disastrous effect on the health of our population had there not been at the same time a general rise in the standard of living with the result that the consumption of bread dropped sharply while at the same time there was a great increase in the

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consumption of butter, eggs, milk, fruits and vegetables other than potatoes (2). Sylvester Graham (1) pointed out in 1837 that fine flour was not injurious provided only small amounts were eaten or much "animal flesh" was consumed at the same time. Osborne and Mendel (3) recently confirmed this idea experimentally and showed it was due to the proteins and vitamins furnished by animal foods such as meat, milk and egg. These foods are now known as "protective foods."

We depend on "protective foods" to supply white bread-eating populations with proteins of high quality, minerals and vitamins. The "protective foods" include eggs, milk and milk products, meat, fish, fruits, vegetables and whole wheat. The "protective foods" with the exception of whole wheat are more expensive than the vegetable foods, and malnutrition has often been found associated with poverty (4).

TABLE 1

Composition of 100 grams

	STONE GROUND "WHITE FLOUR"	ROLLER-MILL BLEACHED WHITE FLOUR
Protein, gram	12.5	10.1
Fat, gram	1.4	0.9
Minerals, gram	1.1	0.4
Calcium, mgm.	44.0	20.0
Phosphorus, mgm.	180.0	92.0
Iron, mgm.	3.3	1.0
Carotene, mgm.	0.2	nil
Riboflavin, mgm.	0.02	0.01
Thiamin, international units	100	10–15

During wars, populations are forced to depend primarily on vegetable foods and wheat becomes of special importance. There are many reasons why this is so.

1. Soldiers need more food than they would require as civilians and their nutrition is of critical importance. They get first choice of "protective foods." Workers in war industries also require more food.

2. Conversion of vegetable foods into animal products or "protective foods" is a very costly process. Only 5 to 10 per cent of the feed fed is recovered as meat and 15 to 20 per cent as milk or eggs.

3. Our Allies, England and Russia, must eat the vegetable foods they raise, and some of the "protective foods" necessary to supplement their diets must come from this country.

4. Wars are destructive both of foods and food producing areas.

5. Efficient farming in war time is impossible because of diversion of manpower from the farm to fighting forces and war industries.

6. Populations of occupied countries and prisoners must be fed.

The United States started the war with a tremendous surplus of food, especially

of cereals. The farmers were urged to increase the production of meat, milk and eggs, and as an inducement the government set prices on these "protective foods" high enough so the farmers could make a profit. The livestock population reached an all-time high and produced animal foods in greater quantities than ever before. Yet there was not enough meat, milk and eggs produced to satisfy the demand with the result that rationing, both voluntary and governmental, had to be resorted to to spread the supply. The surplus animal feeds were soon exhausted. Liquidation of the livestock population was instituted to bring in line the animal population with available feed supplies. The supplies of available "protective foods" are bound to decrease while at the same time demands are increasing. The reduction of available "protective foods" has in fact been confirmed by the President of the United States (115). The decreased intake of "protective foods" must result in an increased consumption of bread. This is characteristic of war food economy. Under such conditions the character of the bread, whether white or whole wheat, becomes of importance in the national food economy and in national health

Dietary surveys before the war have been interpreted to show that large sections of our population are undernourished (5). If such a condition existed with a practically unlimited supply of "protective foods," their decreased intake must obviously cause a deterioration of the nutritional status of the population. If the loss of "protective foods" of animal origin could be replaced by a food of plant origin equally "protective," the nutritive status of the population could be maintained. Fortunately in whole wheat we have just such a food. Since the government has reduced the intake of the "protective foods" of the population by rationing and their removal from civilian food channels, the government bears a responsibility to the people to correct this possible dietary deficiency. It has not chosen to substitute whole wheat for the "protective foods" lost, but instead has resorted to the use of "enriched" white flour.

Under normal circumstances in a free country, the citizen has the right to choose a deficient diet if he wishes and to deal with the consequences in his own way. In war, the government deprives the citizen of freedom to choose foods which he has learned from experience keep him in a fair state of health. Under such conditions the government bears a responsibility to the citizen to help him adjust himself to changing food conditions so he may properly nourish himself. The purpose of this review is to consider this whole question in the light of available evidence. Special attention is paid to experiences in previous wars because of the light they shed on our present situation.

WARS AND NUTRITION. Wars stimulate interest in nutrition. The soldier must be fed properly to win battles. The population at home must be fed adequately to maintain morale and to manufacture the munitions of war. Nutrition has been the concern of military men during war or in anticipation of war.

About 1880 beriberi had become a scourge among Japanese sailors, and fearful of the consequences of incapacitated naval personnel should war break out, Takaki (6) investigated the problem, and found that the disease could be practically eliminated by substituting barley for a large part of the polished rice in the

diet of the sailors, increasing the meat and vegetables, and introducing condensed milk.

J. B. Orr (7) recently recalled that during the Napoleonic wars the men from northern England and southern Scotland who lived in the country and had plenty of whole wheat grain, milk, eggs and vegetables were big, powerful and energetic men who made the best infantry soldiers of Europe. During the Boer war a large percentage of the recruits from this district were short frail weaklings who could not be used as soldiers. A commission was appointed to investigate the cause of this striking change in the physical condition of these men, and the most probable explanation found was that many people had moved off the land and had gone into the slums of the big cities where their eating habits had changed, and they were depending too largely on white flour and sugar.

Wiley (8) pointed out in 1915 that recruiting officers in France, Germany, England and Italy were struck with the growing percentage of men unfit for military service, and the generally accepted cause was deficient nutrition, especially in infancy and childhood. Drummond (2) pointed out that the Italian disaster at Caporetto was in large part due to "more than 7 months' subsistence on inadequate nourishment."

McCarrison (9) after the last war emphasized the rôle of nutrition in gastrointestinal disturbances and emphasized the importance of vitamin B (vitamin B complex had then not been differentiated). He insisted that bad diets consisting of white bread, sugars, margarine devoid of the A vitamin, boiled vegetables and tinned meat are the foundations of gastrointestinal disease (10).

In the present war, gastrointestinal disorders are receiving a great deal of attention in England. Gastrointestinal disorders and functional dyspepsias outclassed in incidence any other group of disorders (11) and accounted for 25 per cent of the cases sent home from the British army in France in 1940. Apparently the disorders of the stomach and duodenum had become more common in medical practice in England during the last few years (11). In very few instances have the gastric disorders originated during military service (12). Apparently most of the patients during civil life had learned to adjust their diets and mode of life to keep their indigestion under control. Many of the acute ulcers were the result of breakdown of old standing ulcers under the conditions of active service (13). Under the strain of heavy air raids there was also an increase in perforated ulcers in the civil population (14).

The vitamin B complex has been reported in clinical studies (15) to be beneficial in gastrointestinal malfunction. In experimental animals gastrointestinal disturbances are associated with deficiencies of the vitamin B complex or its various components (16, 17, 18).

THE RÔLE OF BREAD IN NUTRITION. One of the best treatises on bread is that of Sylvester Graham (1) published in 1837. It is not only very interesting but is applicable to our present day bread problems. In general, his ideas have proven to be scientifically correct. His work is frequently quoted but apparently seldom read, and for this reason will be discussed here in some detail.

Graham (1) emphasized that bread is one of the most important, if not the

most important, articles of diet entering into the food of man. He also emphasized palatability in bread, that from the same wheat kernel can be prepared a most delicious article of diet or "the most miserable trash that can be imagined."

Graham (1) had an unusually good understanding of the nutritive properties of bread. The whole wheat bread was almost a complete food and in addition could cure digestive disorders such as constipation and diarrhea. He warned that the removal of the bran by bolting the milled flour reduced the nutritive value of the "fine flour" obtained.

He (1) quoted a Judge Peters: "Baron Steuben has often told me that the peculiar healthfulness of the Prussian soldiers was in a great measure to be attributed to their 'ammunition,'—bread, made of grain, triturated or ground, but not bolted; which was accounted the most wholesome and nutritious part of their rations."

It is of great interest that the vigor of the German soldier today has also been attributed by Wilder (19) to be due in a large measure to the same type of bread. "The world has been impressed by the terrific might of the German armies. We have witnessed unheard of military efficiency. The German soldier seems to possess unusual capacities. Not only has he been able to endure extreme exertion but his poise and fortitude has been remarkable—Hitler and his henchmen would have us believe that this implies race superiority. I don't believe it.—But Germany early put to work the science of nutrition. The German youth and the German army are well fed. Their bread, Koumissbrot, part rye, part wheat, is made of whole grain flour—the ration calls for a pound and one half a day."

Similarly, Williams and Wilder (20) attribute much of the power of the Russian Army to their whole grain bread. "The Russian army is fed whole grain. Thus far it is the only army to match successfully the whole-grain eating army of the Nazis. The endurance of the Russian citizen equals the vigor of the Russian soldier. The Russian people, eating whole grain bread receive important nutrients denied to people who depend on ordinary white flour for their bread." Yet these authorities did not recommend whole grain for our army or our civil population.

During wars, foods become scarce and bread assumes very great importance in the life of warring nations. During the war between England and France near the close of the eighteenth century, wheat became very scarce in England. Since her experience then has been repeated in World War I and again during this war, it will be described in detail as a quotation by a Mr. Prior (1):

> William Pitt was then prime minister of state, and at his instance the government recommended to the people generally throughout Great Britain to substitute potatoes and rice as far as possible for bread in order to save the wheat for the foreign army. This recommendation was promptly complied with by many of the people. But still the scarcity was alarmingly great. In this emergency parliament passed a law (to take effect for two years) that the army at home should be supplied with bread made from unbolted wheat meal, solely for the purpose of making the wheat go as far as possible.—My father, whom I have often heard talk these things over, was a miller and baker, and resided in the county of Essex, on the border of Suffolk, and near the barracks containing eighty thousand soldiers.

He contracted with government, to supply the eastern district of the county of Essex, with the kind of bread I have mentioned: and he used always to send me with it to the depositories on the day it was baked: and though I was then a youth, I can still very distinctly remember the angry looks and remarks of the soldiers, when they were first supplied with it. Indeed they often threw their loaves at me as I passed along, and accompanied them with a volley of curses. The result of this experiment was, that not only the wheat was made to go much farther, but the health of the soldiers improved so much and so manifestly, in the course of a few months, that it became a matter of common remark among themselves, and of observation and surprise among the officers and physicians of the army. These gentlemen at length came out with confidence and zeal on the subject, and publicly declared that the soldiers were never before so healthy and robust; and that disease of every kind had almost entirely disappeared from the army. The public papers were for months filled with recommendations of this bread, and the civic physicians almost universally throughout Great Britain pronounced it far the most healthy bread that could be eaten, and as such, recommended it to all the people, who very extensively followed the advice:—and the coarse wheaten bread was very generally introduced into families—female boarding schools, and indeed all public institutions. The nobility also generally used it; and in fact, in many towns, it was a rare thing to meet with a piece of fine flour bread. The physicians generally asserted that this wheaten bread was the very best thing that could be taken into the human stomach, to promote digestion and peristaltic action; and that it, more than anything else, would assist the stomach in digesting other things which were less easily digested, and therefore they recommend that a portion of it should be eaten at every meal with other food.

Still, after this extensive experiment had been made with such happy results, and after so general and full a testimony had been given in favor of the coarse wheaten bread, when large supplies of superfine flour came in from America, and the crops at home were abundant, and the act of parliament in relation to the army became extinct, most of the people who had before been accustomed to the use of fine flour bread, now by degrees returned again to their old habits of eating fine bread. Many of the nobility, however, continued to use the coarse bread for a number of years afterwards. General Hanoward, Squire Western, Squire Hanbury and others living near my father's, continued to use the bread for a long time, and some of them still used it when I left home and came to America, in 1816.

In the war of 1914–18, England had a similar experience with shortage of food and wheat again became something precious as an article of diet. The situation was thus expressed by Hopkins (21), "The present shortage in the food supply of the world makes important every detail of knowledge concerning human nutrition.—Particularly desirable now is every scrap of knowledge concerning cereals. Except in Arctic climates, bread and cereals are always important items in the food of mankind, and except where wealth has accumulated and luxury comes in its train, they are by far the most important. . . . Another question arises in connexion with grain foods which are of practical importance at the present time; what is the effect of increasing the percentage extraction of grain on the nutritve value of flour and bread."

In the United States the food situation was so alarming that physiologists drew upon extensive investigations on undernutrition to show that the population could well reduce their weight so that the basal heat requirements would be reduced. In this way less food would be required and important food economies could be made. Lusk wrote (22) "one may reduce the basal requirement of energy by undernutrition, and that this process may largely economize food"—"It may become necessary for Americans to 'train down hard' as people in the

warring nations of Europe are forced to do." In a similar vein Roth (23) wrote "The reduction of diet can safely include all principles, proteins as well as carbohydrates and fats"—"Many would be benefited by dropping occasionally or regularly one meal a day." In fact, loss of weight under some conditions was even considered beneficial. "According to the statistics of life insurance companies a loss of 10 pounds from the average weight of a man of 55 years of age would result in an increase of his value as a life insurance risk" (24).

WAR BREAD—WORLD WAR, 1914–1918. *The Danish experience.* The Allied blockade in 1917 cut off imports into Denmark upon which she depended to feed her large livestock population. Hinhede (25) and Mollgaard were given the task of rationing the food for the country during the blockade. The pigs, competing with the human for the same foods, were killed off. The production of brandy and whiskey was stopped to conserve grains. Rye, which formed the basis of the bread, was milled to 100 per cent and mixed up to 50 per cent with wheat bran which was taken from the cows. Barley, milled to 95 per cent, was also added to bread. "Some doctors were angry and wrote that Hinhede put the people on pig-food and hen-food" (25). In spite of the high fibre content of the bread, the digestibility was reduced only from 94 to 85 per cent (26) and they had available more than twice the amount of bread than they would have had had they milled their grain to 70 per cent. People did not complain nor were there any digestive troubles (26) as a result of the large amount of fibre in the bread. The reason for the success of this program was the ability of the Danish people to make the whole rye-wheat bran mixture into a bread of good quality and palatability. Hinhede (26) pointed out that the Danes had a hundred years of experience in making bread of good quality from such materials. The principal foods of the Danes during this period were this bran bread, barley porridge, potatoes, greens, milk and some butter. The state of health of the people improved. Thus this large scale war experiment proved that wheat bran is a good food.

The English experience. As a result of submarine warfare, England faced a critical food shortage, and to extend their food supply the English had to mill their wheat to a greater percentage, first to 80 per cent and then to 90 per cent. The English were not so fortunate as the Danes since they did not appear to have sufficient experience to bake bread of good quality and palatability with high extraction flours. With characteristic thoroughness the English investigated *a*, the quality of the war bread; *b*, complaints made against the dark bread, and *c*, the nutritional value of the bread, its palatability and its effect on health.

a, The quality of the war bread. The year 1917 when war bread was introduced was a hot year with the result that the war bread was frequently infected with a microörganism resulting in a condition known as "rope" (27). This "ropy" condition was probably favored by the higher nutritive value of the bread, since microörganisms, just as human beings, thrive better on foods of higher nutritional value. War bread contained more moisture than bread of 70 per cent extraction and the added moisture probably also favored the infection of the bread with "rope." This condition was investigated and much information was gained on preventing it.

Because of inexperience in baking bread with flour of 90 per cent extraction, the war bread often lacked porosity and tended to be soggy and doughy and therefore less digestible. Toasting was recommended to make the bread more digestible (28, 29). With experience the quality of the bread improved, but the English did not seem to be able to equal the quality of the Danish bread.

b. The complaints. The complaints against war bread were investigated and Lord Rhondda, the Food Controller, discussed them in the House of Lords (30). Some of the complaints involved digestive disturbances in which any form of bread might be harmful. "Roughly one half of the complaints refer to diseases the course of which cannot possibly be influenced by the kind of bread consumed." "The complaints as a whole largely reflect the fact that the nation is now nearing the end of the third year of unprecedented strain." Discomfort of the hot weather experienced in June was laid to the war bread (30). A feeling of flatulence or fullness was a common complaint (31) and this was ascribed to the soggy doughy bread, the result of inexperience in baking. Looseness of the bowel was another complaint (31) and this was considered an advantage in a nation in which constipation was all too common. Sufferers from digestive complaints tolerated the war bread well (33).

The dark color did not seem to be a frequent cause of complaint. When properly baked the war bread was judged quite palatable and often preferred to the white breads (32).

In certain cases the government granted permits for the use of white flour, but only after careful investigation. "In chronic functional disorders of the stomach (which make up the majority of cases of chronic dyspepsia) the pure wheaten flour can rarely be allowed, for the reason that such cases are so numerous and so protracted in their course that it would be impossible to meet the demands without seriously depleting the wheat supply of the rest of the population" (34).

c. The nutritional value of war bread, its palatability and effect on health. The Food (War) Committee of the Royal Society investigated the English war bread to answer the following questions (32):

1. What gain, if any, in food value to the nation will accrue from a rise in the milling standard from 80 to 90 per cent?

2. What would be the effect on the health of the population of the consumption of bread made from wheat flour of 90 per cent extraction?

3. How far would bread made from such flour prove acceptable to the population?

Digestibility studies were thoroughly and carefully carried out by competent and highly skilled scientists. Three groups of 4 men in each group were the experimental subjects. One group was studied under the supervision of F. G. Hopkins at the University of Cambridge; another, under Noel Paton at the University of Glasgow, and the third under the supervision of J. A. Gardner at the University of London (32). Two breads were compared, one from flour of 80 per cent extraction and the other from flour of 90 per cent. The bread, with one exception, furnished from 45 to 60 per cent of the calories eaten which was more than the customary bread intake.

A slight tendency to flatulence was observed at the beginning by some of the subjects; this later decreased and disappeared. Looseness of the bowels was noted on the 90 per cent bread. The feces always increased in bulk on the 90 per cent bread compared to the 80 per cent. Some of the results obtained are given in table 2.

The losses of energy and nitrogen of the high extraction bread were very small. The gain was roughly 10 per cent in energy and 20 per cent in protein, even when allowance was made for feeding the 10 per cent offal to pigs.

These investigators pointed out that it was difficult to determine the relative contribution of the food and of the gut to the feces, since "the greater part of what leaves the intestine is not the undigested residuum of the food eaten, but material produced in the gut itself." This conception is of the greatest importance, since the nutritive quality of whole wheat and wheat bran was completely misunderstood as a result of digestibility experiments. The larger amount of feces formed in animals fed bran or whole wheat was looked upon as unabsorbed food, leading to the common notion that wheat bran has little or no nutritive value for man. This study directed attention to the possibility that the bulk of the feces did not represent undigested wheat bran but consisted largely of

TABLE 2

	80 PER CENT BREAD	90 PER CENT BREAD
Av. per cent utilization of energy	96.14	94.5
Av. per cent utilization of nitrogen	89.4	87.3
Av. weight of dry feces, gram	156.1	245.0

unabsorbed residues of material produced in the gut. This idea has been fully proven experimentally (35).

The palatability of the war bread was studied with factory workers, 39 men and 8 women. Of these, 20 men and 7 women, all volunteers, ate the experimental bread to the extent of 50 to 60 per cent of their calories for eight weeks. The bread was also made available to other employees. Educated sections of the employees such as managers and foremen were the majority of those who completed the test. The subjects who completed the test agreed the bread was palatable, easily digested and they did not tire of it. When the bread was badly baked the people grumbled. After the experiment was over 61 of the employees signed a petition asking to have the bread made continually available.

There was marked absence of flatulence and complete absence of diarrhea in the case of all who used the bread. The feces in all cases were soft and passed without discomfort. One of the subjects was cured of an acute diarrhea with pain, another of chronic constipation and a third of severe constipation.

The bread was tried out among patients suffering from pulmonary tuberculosis, patients "notoriously capricious in their appetites and easily upset on improper food." The group consisted of 12 men and 13 women who ate the bread for 3 weeks. The women consumed an average of 10.5 oz. of the bread daily and the

men 11.8 oz. The bread was preferred by the majority of patients, all of whom had more or less impaired digestions. No evidence of digestive disturbance was noted in any single case. All the women expressed preference for the bread after using it 3 weeks. Of the men, 8 preferred the special bread, 2 had no preference and 3 either disliked it or preferred ordinary bread. Of the patients that disliked it, all had capricious appetites and appeared to tire of it. Two were febrile cases.

The war bread in England was not an unqualified success, largely because of inexperience in making a good quality palatable loaf, but the nutritive superiority of the bread made from flour of 90 per cent extraction was so impressive that there was some question about continuing such a loaf. Popular demand for the white loaf was, however, great, largely as a result of the unpalatable nature of so much of the war bread the people were forced to eat. The food ministry allowed the return of the white loaf on the grounds that the "offal," or mill feeds, was necessary for the livestock (36). Lancet summarized the situation: "Better bread could have been made with better knowledge, but we deprecate the use of the phrase 'better bread' to describe the return to white flour. Those with a taste for such bread may consider it 'nicer,' but it is elevating personal predilection on a pedestal to make it the standard of goodness. We still read in old books of one man wearing a 'better' coat than another, the words having no relationship to the warmth or the wearing quality of the coat and in this sense perhaps 'better bread' may stand. Of course the *economic* necessity of the transitional period between war and peace may dictate the surrender of 18,000 tons of 'offal' weekly to farmers, pig-keepers and others in order to feed animal stock."

The experience of the United States with war bread was of too short duration to obtain a popular reaction to war bread. Taylor (37) opposed bread from whole wheat flour on the grounds that "the function of the food administration is to secure and conserve food, not treat pre-existing diseases in a compulsory manner, applied to the majority who are not afflicted, as well as the minority who may be distressed, but still possess the right to select their treatment." The current introduction of "enriched bread" (5) involves the rejection of this notion and the acceptance on the part of the government of responsibility for the nutritive quality of the food of the people and the use of the Federal authority, at least in war time, to improve the nutritive level of the population whether they like it or not.

The French had a disagreeable experience with war bread, largely because of the poor quality of the flour used and inexperience in baking a good quality loaf. Apparently the millers did not always co-operate since they sometimes left in the flour "much dust, mildewed and foreign grains, ergot and dodder seeds" (38).

The population in Switzerland accepted their war bread of 87 per cent extraction without too much complaint. Spriggs (31) quoted Professor Feer: "In Switzerland only neurasthenics and hypochondriacs complain." This was probably exaggerated. In war there is apt to be impatience with grumbling even for good causes. Of interest, however, was the experience that hand-fed children

from the third month of age who had white flour in their formulas suffered no digestive disturbance when whole wheat flour replaced the white flour (31).

In Germany the war bread was sour and soggy (26) but the trouble there was that there was not enough of it. Rubner conducted extensive digestibility experiments with various breads and insisted on the basis of fecal losses that highly milled flours were most efficient. His work has been reviewed by Lusk (39). The work involved digestibility studies with special emphasis on the losses of nutrients in feces. The German outlook was analytical and they lacked sound nutritional concepts, yet Lusk (39), who either did not read the English work (32) or did not appreciate it, wrote: "The American faddist who would have incorporated into the permanent law of the land the compulsory production of whole wheat bread and its infliction upon the population meets here his nemesis. The British people during the war felt that whatever else happened, they would return to white bread as soon as they possibly could. Here instinct, supported by Science, triumphs once again over ignorance and bigotry." Science has not supported this contention of Lusk but has completely disproved it. The composition of feces was an insufficient basis for judging the nutritive value of bread or any other food.

Out of the war came the first thorough-going study by Osborne and Mendel (3) of the nutritive value of the wheat kernel, with the rat as the experimental animal. They established the following:

1. The proteins of white flour are inferior to those of the wheat germ or wheat bran.

2. The vitamins are associated with the proteins of high quality in the wheat germ and wheat bran.

3. Meat, milk or eggs when furnishing one-third of the proteins consumed will adequately supplement the white flour proteins.

As our knowledge of nutrition advanced, especially with a better appreciation for the rôle played by proteins of high quality, minerals and vitamins, our understanding of the effect of milling on the nutritive properties of wheat increased.

For thousands of years wheat and rice have figured prominently in the diets of the bulk of the human population. The whole grain cereals provided adequate amounts of the vitamin B complex, proteins of good quality, minerals and other essential factors such as vitamin E, carotene, the essential unsaturated fatty acids, and perhaps many as yet unidentified factors. The whole grain cereals needed supplementation with either meat (including some bone), milk or fish and some fruits or vegetables. Apparently this could readily be accomplished. With the advent of modern milling processes, the germ and outer coatings of the wheat berry are removed from the flour. With these "offals" or by-products are lost the bulk of the vitamin B complex, most of the important minerals including iron and calcium, most of the fat including vitamin E and a larger part of the protein of high biological value, leaving in the white flour mostly carbohydrates and proteins of lowered quality (3). The wheat was milled in this way because people wanted white flour, and because of the greater stability of white flour toward rancidity changes after the removal of the fat. Moreover,

insect pests such as the flour weevil did not thrive so well in white flour as in whole wheat flour because of the removal of nutrients required by the flour weevil (40). Instead of being alarmed at the decreased nutritive value of white flour as shown by the inability of insect pests to thrive on it, the production of white flour was hailed as a great forward step. Nutrition at the time had not progressed to the point where the true nature of the phenomena observed was fully appreciated. During the intervening years, the high nutritive qualities of whole wheat bread became increasingly apparent as the evergrowing number of factors resident in the whole wheat became known. The nutritional poverty of white bread as compared with whole wheat bread was so great as to demand attention under the impact of war when it was realized that the nutrition of the army and civilian population would play a major rôle in the struggle for survival. The bread question became prominent both in England and America and received a lot of attention.

World war ii, 1939–. *The English experience with fortification of white bread.* The discussion on fortification of white bread in England was opened by Mr. Boothby (41), parliamentary secretary to the Ministry of Food, when he announced in parliament that white flour was to be fortified with thiamine. The reason he gave for fortifying the white flour was to meet the objection against white flour by nutrition experts, who stressed the superior nutritive value of whole wheat flour as compared with white flour. The government had not suspended the manufacture of white flour because the great majority of consumers preferred white bread and because the keeping qualities of white flour were definitely greater than those of whole meal flour. "To overcome the objection that white flour was lacking in vitamin content, it had been decided to fortify it with vitamin B_1" (41). Whole wheat bread was to be available at the same price as white bread.

This program was endorsed by Moran and Drummond (42) who pointed out as an additional objection to whole wheat bread that it would reduce the available wheat by-products for the stock industry and "in particular, jeopardize our milk supplies." In their paper they stated, "The introduction of the new white flour will undoubtedly stultify the controversy of white versus brown bread, since it is mainly in respect of vitamin B_1 that the white loaf has been open to attack. There is not the same evidence that we are deficient in vitamin E or members of the vitamin B_2 complex which are present in whole meal flour. . . ." In this he proved to be wrong because the controversy became more intense than ever.

A leading article in Lancet (43) reviewed the whole question and gave the following arguments in favor of fortifying white flour with thiamin.

1. People do not like brown bread but pigs and chickens like and need the "milling offals" when white flour is made.
2. Brown bread needs much more yeast to bake than white bread.
3. Brown bread has usually been more expensive than white.
4. Under war conditions involving storage problems, white flour is preferable to whole wheat flour because it keeps better.

5. By ordering that whole wheat bread shall be available at the same price as white, the government removed the grievance of those who complain that they do not eat brown bread because it is too expensive.

6. By the addition of thiamin to white flour, the most serious vitamin deficiency of white flour is made good.

7. The average daily bread intake in England was 0.5 pound per capita. The consumption was however unevenly distributed, better-off people averaging about 4 ounces a day and working people as high as 1.5 pounds. It is these working people with low intake of the expensive "protective foods" such as milk, cheese, eggs, fruits and vegetables whose diet will be "highest in carbohydrate, and who will most benefit by fortification with added vitamin" (42).

Thus was ushered in a revolutionary change in human life—the acceptance by government of responsibility for the nutritional welfare of the population. It was unfortunate that with respect to the bread, the food staple of the common people, the program started with the uncertain assumption that by adding thiamine to white bread, its nutritive value when combined with the remainder of the diet available to the common people, has been restored to that of whole wheat flour. This necessitated the assumption, based on no evidence, that the English diet which included white bread as the staple food was deficient only in thiamine.

The program of fortifying white bread with thiamine did not go unchallenged. Sir Graham-Little (44) pointed out that it was proved by statistics that the health of the nation during the last part of the great war and in the period that followed was better than it had been before and it was attributed in large part to the whole wheat bread the English were obliged to eat as a result of the war.

The Medical Research Council in a special memorandum (45) immediately called attention to the importance of the vitamin B_2 complex in whole wheat bread, and that it could not be ignored even though the knowledge on this subject is less exact than for vitamin B_1. The memorandum also called attention to the loss from white flour of important fat soluble vitamins, minerals, and protein of high quality. They recommended under-milling, which would result in the extraction of 85 per cent of flour from wheat, in place of the usual 72 per cent, thereby saving the bulk of the nutritive value of the wheat berry without including any appreciable amount of the rough bran.

Thus, the controversy of white bread versus brown bread was started and it was discussed in the *Times*, in the trade journals and medical journals. The discussion became intense, bitter and acrimonious. The nutritional aspects of the discussion were well mirrored in Lancet, and will be discussed in detail because of the great importance it was bound to have on nutritional thought.

In a second leading article in Lancet under the heading "Second thoughts on bread" (46), the discussion was still largely on the basis that the thiamine content of the wheat berry is the factor to be concerned with in the choice between white flour and whole wheat flour.

Graham-Little (47) attacked the thiamine fortification program and considered

the minister's objections to whole wheat bread in the light of the nation's experience in the last war. He discussed

1. *Alleged indigestibility of whole wheat bread.* Graham-Little referred to the report of the Committee of the Royal Society "On the digestibility of bread" (32). This report showed that there were no symptoms of indigestibility on whole wheat bread in healthy subjects or even in invalids.

2. *Alleged unpalatability.* Only a small minority, 8 per cent, expressed a preference for ordinary bread after a trial was made of both breads. The "invincible preference" for white bread is a myth.

3. *Keeping qualities.* He questioned whether the difference in keeping quality is such a great factor as to prohibit the use of whole wheat flour.

He suggested that the resistance to whole wheat bread may come from the flour milling industry, a giant trust, who were safeguarding their 100 million dollar investment in milling equipment.

In Parliament (48) it was brought out by a question from Fremantle that all medical men consider whole wheat bread "infinitely better" than fortified bread. The parliamentary secretary replied that the really primary reason for the fortification of white bread with thiamine was the large number of people who did not care to eat brown bread.

Graham-Little (49) attacked political considerations which result in approval by default and quotes from the Penquin Book, *Science in war* on advisory bodies, "They seem to have been much more concerned with agreeing with government decisions than with challenging them in the name of science. . . . In the long run the 'yes-men' of science are likely to be at least as dangerous as the 'yes-men' of politics."

Franklin Bicknell (50) urged the parliamentary minister, "If the minister knows, as he must, that stone-ground flour is best for the nation he should say that B_1 in bread will help slightly, but that he cannot provide really valuable stone-ground bread because of the millers."

In a survey by the Ministry of Food's scientific adviser to determine the relative popularity of brown and white bread with the public (51) the following information was brought out. In the poorer areas, 41 to 46 per cent of the public ate some quantity of brown bread regularly and in the richer areas the figure was 65 per cent. Brown bread was actively disliked in about 34 per cent of cases in all classes. If brown and white bread were the same price about 28 per cent of the poorer people and 13 per cent of the richer people would buy more brown bread.

Added impetus was given to the controversy when Chick (52) showed by feeding experiments with rats that white flour enriched with thiamine was decidedly inferior to whole wheat flour. The rats on whole wheat flour gained about twice as much weight as those on "enriched" white flour, and they utilized their food more efficiently. The rats on the whole wheat flour diet made 1 gram of gain with a food intake of 2.47 grams while it required 3.02 grams of the "enriched" white flour diet to make the same gain. When the diets were switched, the rats transferred from the "enriched" white flour diet to the whole wheat

diet made an immediate spurt in growth while those transferred from the whole wheat flour diet to the "enriched" white flour diet suffered an immediate check in growth.

A leading article in Lancet (53) immediately took cognizance of this new information. It pointed out that considerations of the inferiority of white flour can no longer be concerned only with vitamin B_1 but with other factors, the nature of which was not very certain. Lancet now took the stand that the "signpost points to whole meal as the high-road to better health and greater food economy." "Political or economic considerations may make it impossible to take this road in wartime, but the general conclusion from the nutritional standpoint is not thereby affected."

Graham-Little (54) inquired of the food minister in Parliament whether Doctor Chick's work had any influence on the policy to fortify white bread with thiamine and the answer was in the negative.

As a result of the controversy to date the British Government modified its bread policy which Lord Woolton (55) announced in the House of Lords. He had been impressed by the unanimity of scientific opinion on the nutritive value

TABLE 3

FLOUR	FIBRE	ASH	PROTEIN	VITAMIN B_1	CALCIUM	PHOSPHORUS
	per cent	*per cent*	*per cent*	*international units per gram*	*mgm. per 100 gram*	*mgm. per 100 gram*
I. 85%	0.60	0.90	11.4	1.20	27	203
II. 85%	0.85	0.94	11.5	1.15	27	211
White, 73%	0-0.2	0.46	10.6	0.35	15	101
Wholemeal, 100%	1.8	1.51	11.9	1.40	36	343

of wholemeal bread. He considered it the proper attitude of the government to make wholemeal or national wheatmeal (85 per cent extraction) available in adequate supply to the public at the same price as white bread, and at the same time to draw the attention of the public to the advantages of brown breads. The government would at the same time continue its efforts to produce a white bread fortified with thiamine.

Willcox (56) pointed out that in Mesopotamia in the last war, a loaf made from 75 per cent whole wheat meal and 25 per cent white flour proved to be very palatable. Wholemeal bread properly baked has more flavor than white bread and he did not consider the universal use of whole meal bread an insuperable problem.

On May 22, 1941, the Medical Research Council (57) issued a second memorandum on national wheatmeal flour in which they defined more exactly the character of the 85 per cent wheat meal which they recommended (table 3).

They recommended flour I with only 0.6 per cent fibre. Since that would require modification of milling machinery, they recommended the interim adoption of flour II containing not less than 1 international unit of thiamin (3 micro-

grams) per gram nor more than 0.9 per cent fibre, assuming a water content of 15 per cent. They recommended the addition of calcium to neutralize the effect of phytic acid which diminishes the availability of the calcium present in the diet. They opposed the addition of iron because of its possible destructive catalytic effects. "On technical and aesthetic grounds, the loaf baked from such flour was excellent." In a leading article, Lancet (58) pointed out that the recommendation was the result of "a most careful scrutiny of the nutritive value of different parts of the wheat grain." They strongly recommended the bread but noted "Against the change can only be set popular prejudice, (much of it will go when everyone has sampled the national loaf) the better keeping qualities of white flour and an understandable reluctance to scrap the machinery which is now producing vitamin B_1 cheaply and on a large scale."

TABLE 4

WHEAT IMPORTS	HUMAN FOOD	AVAILABLE CALORIES	DIGESTIBLE PROTEIN	AVAILABLE		THIAMIN	RIBOFLAVIN	NICOTINIC ACID
				Calcium	Iron			
10^6 tons	*10^6 tons*	*10^{12}*	*10^6 tons*	*tons*	*tons*	*10^{12} i.u.*	*tons*	*tons*
6	Flour, 75%..... 4.5	16.11	0.45	450	58.5	2.025	2.25	67.5
	Milk from 1.5×10^6 tons wheat feed.. 3.43	2.23	0.109	4,120	4.1	0.79	5.15	15.1
	Total.....	18.34	0.559	4,570	62.6	2.815	7.40	82.6
5.3	Flour, 85%..... 4.5	14.22	0.446	−45	90	4.05	4.50	203
	Milk from 0.8×10^6 tons wheat feed.. 1.83	1.19	0.058	2,200	2.2	0.42	2.75	8.1
	Total.....	15.41	0.504	2,155	92.2	4.47	7.25	211.1
	Change in total nutrients available with 700,000 tons shipping space saved.....	−2.93	−0.055	−2,145	+29.6	+1.65	−0.15	+128.5

The proponents of fortification opposed the national wheatmeal (85 per cent extraction) on grounds of non-acceptability by the British public and because the change over from a 75 per cent extraction of the wheat berry to an 85 per cent would so sharply reduce milk supplies as a consequence of the loss of wheat feed for the cattle, that there would be a net loss in important nutrients such as digestible protein, riboflavin and available calcium. This thesis was first presented in great detail by Wright (59) and was subsequently critically reviewed by Bacharach (60) who corrected some minor errors in Wright's paper (59), but reached essentially the same conclusion the details of which are given in table 4.

The following conclusions were drawn from the table.

1. There was a saving of 700,000 tons of shipping space when the same amount of flour, namely, 4,500,000 tons, is obtained by milling wheat to 85 per cent instead of 75 per cent.

2. The loss of milk as a result of the decreased wheat feed for cattle added to the lower digestibility of 85 per cent flour (digestible protein—white flour, 91 per cent, national wheatmeal, 86 per cent; digestible carbohydrates—white flour, 94 per cent, national wheatmeal, 89 per cent) would result in the following changes.

a. Loss of 55,000 tons of digestible protein
b. Loss of 2,145 tons of available calcium
c. Gain of 1.65×10^{12} I.U. of thiamin
d. Gain of 128.5 tons of nicotinic acid
e. Gain of 29.6 tons of available iron
f. Loss of 2.93×10^{12} of available calories
g. Loss of 0.15 ton of riboflavin

Kent-Jones (63) weighed the gains against the losses, and urged that the losses of digestible protein, calcium and riboflavin far overbalanced the gains of thiamin, iron and nicotinic acid. The gain in nicotinic acid he argued is of no importance since the average diet already has more than adequate amounts. The gain in thiamin is also of no importance since white flour was to be fortified with thiamin. He concluded "the case for national wheatmeal is finally and definitely exploded on sound scientific grounds."

Fraenkel (64) criticized Bacharach's table (60) on the ground that he jumbled shipping space with nutritional values. If shipping space were the prime consideration, it would be useless to talk about nutritional values. If nutritional values were concerned, the whole question must be put on a nutritional basis. If 6,000,000 tons of wheat were milled to 75 per cent, 4,500,000 tons of white flour would be obtained plus 1,500,000 wheat feed ("offal"). By milling to 85 per cent, 5,100,000 tons of wheatmeal flour would be obtained and only 900,000 tons of wheat feed. Fraenkel pointed out that for proper consideration, only 4,500,000 tons of 85 per cent wheatmeal flour should be milled from 6,000,000 tons of wheat leaving 1,500,000 tons of wheat feed "offals" and unmilled wheat combined to be fed to cattle. In this way, the same amount of milk would be obtained regardless of the flour produced and no shipping space would be involved. The difference in nutrients available between milling 75 per cent white flour or 85 per cent wheatmeal would be the result of the difference in digestibility of 75 per cent flour and 85 per cent wheatmeal. Using Bacharach's data, Fraenkel recalculated the changes in nutrients from the change over to 85 per cent wheatmeal. In each case, 4,500,000 tons of flour would be obtained and the same amount of milk, namely, 3,430,000 tons.

On this basis, as a result of the change over from 75 per cent to 85 per cent flour, there would be little change in digestible protein, the loss of available calcium would be cut down, and there would be no loss but a gain in riboflavin. The gains in the other nutrients would be enhanced (table 5).

The argument against 85 per cent wheatmeal because of decreasing the milk supply apparently did not carry very much weight. It was widely discussed pro and con. E. Lester Smith (65) pointed out, "Dr. Wright cannot have his bread and let the cow eat it." He favored 85 per cent wheatmeal to improve the

health of the population, and even favored if necessary the feeding of the white flour to cows to maintain the milk supply. Lancet (66) pointed out that "the cow was not a 'penny-in-the-slot' machine into which you insert a production ration of any given size and automatically get back the exact equivalent of your feeding stuff as milk."

Chick's paper on the superior nutritional value of national wheatmeal over "enriched" white flour led the proponents of fortification of white flour to search for experimental evidence to support their case. This was soon forthcoming in a paper by Wright (67) who criticized Chick's work (52) because the food intakes of the rats receiving "enriched" white flour and national wheatmeal were not equalized, the wheatmeal being consumed in greater quantity, thus accounting for their superior growth and well being. Doctor Wright argued that 85 per cent wheatmeal was more palatable to rats than white flour, and since no way was known to equalize the palatability of the two flours, the rats receiving the national wheatmeal were fed only so much food as the rats on "enriched" white flour consumed. Under these conditions there was little difference in

TABLE 5

	AVAILABLE CALORIES	DIGESTIBLE PROTEIN	AVAILABLE		THIAMIN	RIBOFLAVIN	NICOTINIC ACID
			Ca	Fe			
Change in total nutrients available resulting from a change to 85% flour from 75% flour.....	-1.89×10^{12}	−4,000 tons	−305 tons	+31.5 tons	$+2.025 \times 10^{12}$ I.U.	+2.25 tons	+135.5 tons

growth of the rats. The experiment was repeated with the two breads fed along with other foods so that the total would represent the ordinary mixed human diet. The diet consisted of bread 35.5 per cent, boiled potato 30.0 per cent, cooked meat 6.0 per cent, fresh milk 3.0 per cent, cooked fish 3.0 per cent, margarine 3.0 per cent, cheese 0.7 per cent, cooked vegetables 14.0 per cent, jam 1.2 per cent, eggs 1.2 per cent and sugar 2.4 per cent. On the mixed diet containing the national wheatmeal the rats were somewhat superior, even though limited to the same food intake voluntarily consumed by the rats on the "enriched" white flour diet. However, her work was not confirmed by similar and more extensive studies (107) which instead supported Chick's work.

Though the government (55) announced that national wheatmeal would be available in abundance, a hitch developed somewhere and it was not done. The question was raised in Parliament (68) and Lancet (69) demanded an investigation to locate the obstruction, whether it was with the millers, master bakers or the public.

During this period a very revealing American article on bread appeared. Pewters, Mason and Higgins (70) compared whole wheat flour with "enriched" white flour when added to a basal diet which was representative of that of thou-

sands of Americans. It included in addition to bread which was the only variable, butter, skim milk powder, cornflakes, beef, potato, cheese, polished rice, gelatin, apple jelly, string beans, carrots, peaches, pears, sugar, candy and cake or cookies. Thus, any results obtained would, in so far as the diet was concerned, be applicable to human beings. Four types of flour were compared: white flour, white flour fortified with thiamine, white flour fortified with thiamine and riboflavin and wholewheat flour. The rats grew a little better with thiamine enriched flour than with white flour, a little better still with flour enriched with thiamine and riboflavin, but decidedly better on whole wheat flour. Not only did the rats on the whole wheat flour grow more but their appearance was improved. They concluded that flour must be enriched with factors other than thiamine and riboflavin to obtain a flour of nutritive quality comparable to that of whole wheat flour. After some uncertainty in the interpretation of these data (102) the workers in this laboratory provided additional evidence (116) which showed conclusively that whole wheat bread is nutritionally superior to "enriched" white bread.

TABLE 6

Average intake of plentiful foods consumed in 1935 and 1941 in ounces per week

FOOD	WOMEN, 1935	WOMEN, 1941
Bread	35.2	45.6
Flour in puddings, etc	3.9	5.1
Oatmeal	0.5	1.6
Proprietary cereals	0.8	1.4
Potatoes, cooked	21.1	33.0
Green vegetables, cooked	9.2	13.9
Root vegetables, cooked	4.7	7.4
Legumes, cooked	2.8	2.4

The importance of bread in the English dietary under wartime conditions was emphasized by a comparative study of middle-class diets in peace and war (71) as shown in table 6.

Since so large a part of their food consisted of bread, the protective factors in the whole wheat bread were of great importance.

The acceptance of the national wheatmeal bread was unsatisfactory and Lancet in a leading article (72) inquired into the causes. It pointed out that "never before had the knowledge been sufficient to select with confidence a loaf which would look and taste almost like white bread and yet contain such a large share of the best nourishment of the whole wheat grain." The Medical Research Council had recommended the preparation of a bread by undermilling to 85 per cent which would give a uniform product of satisfactory appearance and taste. The instructions of the Ministry of Food to the millers was so worded, however, as to allow the mixture of 15 per cent bran with white flour and still meet the specifications of the Food Ministry. The result was lack of uniformity in the national wheatmeal flour which was responsible in part for its lack of

acceptance. Lancet (72) observed "In this conflict between the national and individual interests, the millers have thus won the day, and a golden opportunity of furthering the health of the people was lost."

The character of the national wheatmeal loaf became the subject of a heated controversy. In Parliament Profumo (73) asked the parliamentary secretary to the Ministry of Food on what grounds some of the recommendations of the Medical Research Council on the national wheatmeal loaf were turned down. The parliamentary secretary answered that none of the recommendations were rejected, whereupon Profumo told the secretary he did not know what he was talking about.

Graham-Little (74) suggested one of the reasons for bungling the program for an increase in consumption of long extraction flour is the influence exercised by the milling industry, with its formidable occupation of key positions in the Ministry of Food. He urged the medical profession to lead the revolt against the Food Ministry.

Graham-Little (75) pointed out that the recommendations of the Medical Research Council limiting the wholemeal flour to 0.9 per cent crude fibre with 1.0 international unit of thiamine per gram was largely ignored. As a result, a variable and poor quality loaf was produced and in spite of the expenditure of £29,000 in advertising, the consumption of national wheatmeal was about 7.0 per cent. He (75) sponsored a motion in the House of Parliament to appoint a committee to find out what are the considerations which obstruct the exclusive provision of a national wheatmeal loaf of the highest possible extraction. "The point of the demand is that it is feared that the reasons operating against the production of this type of loaf are not based upon the science of nutrition but upon the interests of commercial firms."

The whole controversy ended on March 11, 1942, when Lord Woolton (76) announced in the House of Lords, "His Majesty's Government have therefore decided to increase to 85 per cent the ratio of flour from milled wheat in this country. This means we shall stop the production of white bread." It was hoped the same bread would soon be supplied to the army. The decision was welcomed. Lord Hankey "hoped the millers and bakers would play their part in producing a palatable bread all over the country." "So the matter ended for the time with victory for science because it had an economic ally in shipping."

It should be emphasized (77) that the change to 85 per cent national wheatmeal flour was not the result of shipping considerations only, but saving of shipping space tilted the balance in favor of national wheatmeal.

Leading articles in Lancet (78) and the British Medical Journal (79) greeted the decision with much satisfaction. The British Medical Journal summarized the controversy, "Thus comes to an end a controversy that has lasted for 2½ years, and on which many hundreds of thousands of words have been spent in speech and print. As the public accepted the standard loaf in the last war without much ado, it has been difficult for anyone outside the Ministry of Food to understand why the liberty of the subject in respect of white bread has been looked on as such a precious thing. During the past three months as the columns

of the *Times* have shown, criticism of that Ministry's policy has become progressively acute, and Sir Ernest Graham-Little has attacked it in the House of Commons, this time to good effect.—There has been a suspicion in more than one quarter that the interests of the millers have been allowed to stand in the way of the nation's health, and until this moment the Ministry of Food has shown signs of falling short of whole-hearted advocacy of its wheatmeal loaf.—It is a punishable offense to water milk and dilute the solids in it. Why, then, should it be thought praiseworthy to remove from the wheat berry the valuable minerals and vitamins it contains."

One of the results of the controversy was a reinvestigation of the digestibility of wholewheat bread and the biological value of the proteins of various parts of the wheat berry. Drummond (80), acting as scientific adviser to the Ministry of Food, suggested that the branny layers of the wheat berry, even when finely ground, might be injurious to some gastrointestinal tracts. A wheat flour was chosen which had removed from it the more indigestible fractions, but retained the bulk of its minerals and vitamins. It was largely on digestibility trials that it was decided to go for an 85 per cent flour. This was immediately challenged

TABLE 7

EXTRACTION OF WHEAT	DATA USED BY WRIGHT		DATA OF KREBS AND MELLANBY	
	Utilization			
	Protein	Calories	Protein	Calories
per cent	*per cent*	*per cent*	*per cent*	*per cent*
75	89	92	91	97
85	81	87	89	94

by Scott (81) who insisted that "there is not a scrap of published evidence that an 85 per cent extraction flour is more digestible than whole-wheat meal." He referred to Drummond's claim as outrageous.

Krebs and Mellanby (82) reinvestigated the utilization of calories and protein of white flour and national wheatmeal and found the differences less than those quoted by Wright (59) who argued that the losses from decreased digestibility would balance the additional flour gained from an 85 per cent extraction, and that nothing would be gained by the change to 85 per cent wheatmeal from white flour (table 7).

On the basis of the figures of Krebs and Mellanby (82), there is little difference in digestibility (utilization) of white flour and 85 per cent extraction flour.

A most revealing communication on this subject was published by Chick (83) who investigated by the growth method with rats the biological value of the proteins of whole wheat flour, 85 per cent extraction flour and white flour. The proteins of 85 per cent extraction flour showed an advantage in growth of 13 to 16 per cent over the proteins of white flour, while whole wheat flour showed an advantage of 17 to 26 per cent. The utilization (digestibility) of whole wheat flour and national wheatmeal was less by 6 per cent and 3 per cent respectively.

Thus, the superior biological values of the proteins of the outer layers of the wheat berry more than offset the greater digestibility of the proteins of white flour.

The investigations of Borgstrom (35) have done much to enable us to understand Chick's results (83) and have clearly shown how erroneous they were who held that wheat bran had little value or was worthless to the human because of its alleged indigestibility. He (35) showed that *in vitro* as much as 90 per cent of the nitrogen of wheat bran could be brought in solution by the action of pepsin and trypsin. The nitrogen could be removed from the intact cells by these enzymes without the necessity of rupturing them. The larger part of the nitrogen in the feces of bran fed animals originated not in undigested wheat bran proteins, but in the digestive secretions which were stimulated above normal by the bran and in the bacteria. This nitrogen exists in a state largely unavailable to the organism. Wheat bran increased the fecal output as well as the peristalsis in the gut.

The advantages of the increased quantity of digestive juices was an increase in the rate and intensity of digestion, thereby increasing the amount of nutrients released from the wheat bran for the nutrition of the organism. These nutrients apparently compensated for the loss of fecal nitrogen arising from the failure of these digestive secretions to be reabsorbed with the result that the animal retained more nitrogen for physiologically productive purposes as shown by Chick (83). These results were obtained with rats and with men as the experimental subjects. Even after about 60 per cent of the wheat bran was removed by previous in vitro digestion the residue was still able to reduce the loss of nitrogen in the urine.

The calcium of the wheat bran was found to be readily utilizable (35) but it was apparently offset by the loss of calcium in the digestive secretions. The alleged unavailability of the calcium of wheat bran can be explained by the losses of calcium in the digestive juices which fail to be reabsorbed.

In the light of the evidence now available, the English have been too conservative in milling their wheat to 85 per cent and discarding the bulk of the bran. The value of bran in the human diet has been established by the mass-experiment of Hinhede (26) in Denmark, and proven scientifically by the investigations of Osborne and Mendel (3), Borgstrom (35) and the Food (War) Committee of the Royal Society (32).

THE CANADIAN EXPERIENCE. McHenry (84) in 1940 compared "enriched" white bread with whole wheat bread and found the latter greatly superior. The Canadian medical men and nutritionists early appreciated the superiority of whole wheat bread over "enriched" white bread and their leading scientists, among them McHenry, Tisdall and Best (105) took a definite stand against fortification of white flour. They insisted that the way to get the vitamin B complex in bread was to so mill the wheat that these vitamins would be retained. The millers and scientists co-operated in extensive milling experiments with some interesting results. They controlled their milling operations by thiamin analyses, on the assumption that if thiamin were retained, "the attendant members" of

the vitamin B complex would also be retained. They found great variability in the thiamin content of their wheat.

One of the interesting results of their investigations was the difference in the thiamin distribution in the wheat kernel. By breaking the kernel transversely, they obtained three fractions, the germ with 3300 I.U. per pound, the germ end without the germ with 915 I.U. per pound and the brush end with 252 I.U. per pound.

By decreasing the moisture content of the wheat milled from 15.5 per cent to 12.5 per cent, they could get a white flour with the retention of a fairly large proportion of the vitamins of the wheat berry. As a result of a rather extensive series of investigations of these nutritionists in co-operation with the millers, a white flour of 75 per cent extraction was produced with 363 I.U. of thiamin per pound as compared with 254 I.U. in the white flour made in the ordinary milling process (15.5 per cent moisture) (105).

Taking advantage of these investigations the Canadian Government issued the following specifications for two types of flour and two types of bread.

1. Vitamin B white flour shall contribute not less than 400 I.U. of vitamin B_1 "with the other members of the vitamin B complex in the quantities associated with this amount of vitamin B_1 in the wheat from which the flour was produced."

2. Vitamin B flour shall be flour other than white flour that will contribute per pound of flour not less than 550 I.U. of vitamin B_1 together with the attendant members of the vitamin B complex.

3. Vitamin B white bread shall be baked solely from vitamin B white flour with 4.0 per cent non-fat milk solids and which shall contain not less than 220 I.U. of vitamin B_1 per pound together with the attendant members of the vitamin B complex.

4. Vitamin B bread shall be bread made solely from vitamin B flour with not less than 4.0 per cent non-fat milk solids and shall contain not less than 300 I.U. of vitamin B_1 per pound together with the attendant members of the vitamin B complex.

THE AMERICAN EXPERIENCE. Compared to the way the English treated their bread problem, the American performance seems below par. Unlike the English, they have not discussed with any degree of thoroughness the relative merits of whole wheat bread, white bread and "enriched" white bread. The whole question of fortification of foods including bread was first discussed at a symposium held at the meeting of the American Institute of Nutrition at Toronto, April 1939. Morgan (85) discussed the difficulties and uncertainties of the problem and warned that " 'Proceed with caution' must remain the watchword still." Her subsequent investigations (137) justified this cautious attitude since she found with rat feeding tests that whole wheat bread was superior to "enriched" white bread. Moreover, because of the large intake of bread, whole wheat bread proved to be a slightly better source of the vitamin B complex than about 10 per cent yeast.

Roberts (86) discussed the nature of the American diet and showed the multiplicity of food factors removed in the milling of wheat. She cautioned that "if

the plan is accepted, the exploitation of the products should be so safeguarded as not to give the impression that all the deficiencies of the cereal have been overcome." She considered fortification a "first-aid" measure and said, "We must remember that the last word has not yet been spoken in respect to nutrition and that there may still be many essential dietary factors of which we are as yet unaware. We cannot, therefore, trust solely to fortification, but must still put our major faith in a varied diet containing generous amounts of natural foods."

The most outspoken against fortification was Sebrell (87) who said, "Finally, to me it seems a little ridiculous to take a natural foodstuff in which the vitamins and minerals have been placed by nature, submit this foodstuff to a refining process which removes them, and then add them back to the refined product at an increased cost. Yet this seems to be the thing that is being proposed. If this is the object, why not follow the cheaper, more sensible, and nutritionally more desirable procedure of simply using the unrefined, or at most, slightly refined natural food." Sebrell (95) later reversed himself and actively supported the "enrichment" of white flour. Taylor (88) suggested, "We should first seek retention of native vitamins."

In spite of this conservative and uncertain attitude toward fortification of foods, including the refinement of cereals, the Food and Nutrition Board of the National Research Council announced on January 29, 1941 its recommendation to enrich flour and bread. Thiamine and nicotinic acid were essential additions to claim fortification. Riboflavin was optional, mainly on account of its unavailability.

The proponents of fortification of flour with thiamine and nicotinic acid freely admit the superiority of whole wheat bread, but insist that a start should be made with "enriched" white flour while the public would be educated to the advantages of whole wheat flour.

The Council on Foods and Nutrition of the American Medical Association (89) accepted the "enriched" white flour because "thiamin is the component which makes whole wheat most significant in the diet at the present time." No attempt was made to justify this sweeping statement. The Council pointed out the presence of factors other than thiamine in whole wheat not present in white flour, but this objection to white flour was conveniently sidestepped by calling these other factors "plus values" which the Council assumed without any supporting evidence, was furnished by the rest of the diet. Yet they recognized that the "rest" of many poorer diets might not furnish these "plus values." Finally, they emphasized the high nutritional qualities of whole wheat and recommended undermilled flours. Thus the Council on Food and Nutrition straddled the issue of whole wheat flour versus "enriched" white flour and failed to give the country the leadership so ably given to England by the British Medical Council. Moreover, direct experimental evidence proved (70, 116) that the "plus values" were not present in the diet of large sections of our population. The American Medical Association ignored the controversy over white and brown bread in England and the progress resulting from experimental investigations. One year after the National Nutrition Conference in an editorial (113)

a whole chapter was devoted to "enriched" bread without any reference to events or progress in the intervening year. Two years after the National Nutrition Conference, the Council on Foods (114) still emphasized thiamine and to a lesser extent riboflavin, in discussing "enriched" bread, no mention being made of accumulating literature on the inferiority of such enrichment compared to whole wheat bread.

In May 1941 the National Nutrition Conference for Defense met at Washington to discuss nutritional problems created by the war emergency. "Enriched" white flour was accepted without discussion. The relative merits of "enriched" flour versus whole wheat flour as a national nutritional problem merited more consideration than it received. Murlin (5) gave his consent "to this proposal of enrichment rather reluctantly," and pointedly remarked, "Six such factors are now available in chemically pure form, and without the achievements of the synthetic chemists working with the nutritionist we could not embark on the new program of vitamin enrichment of flour, shall I say for better or for worse?" He also used the occasion to point out:

1. Dogs fed the six synthetic vitamins remained deficient but the deficiency cleared up at once when the dogs were fed yeast, indicating essential, but as yet unknown factors in the vitamin B complex. Digestion was notably improved by the addition of yeast.
2. Pantothenic acid has been shown to have a favorable effect on digestion.
3. Whole wheat bread would result in a great saving of food, in addition to other valuable factors.
4. Studies made in Switzerland seemed to show that soldiers living on whole wheat bread maintained their fitness better than soldiers living on white bread.

In place of critical discussion and experimental work there appeared a large volume of literature, monotonously similar, with the apparent object of proving to the American people that they will not eat whole wheat bread. The unwillingness of people to eat whole wheat bread has been overemphasized. Tobey (90) maintained that the average consumer is not keen about whole wheat bread as a steady diet, even if it is to his advantage to consume it. "It has always been so, and probably always will be, especially since our white breads are now virtually equivalent in nutritive properties to 100 per cent whole wheat. The bakers and millers, by 'enriching' bread and flour, have succeeded in accomplishing what Mr. Graham tried so vociferously, but failed so dismally to do." It is not true that "White breads are now virtually equivalent in nutritive properties to 100 per cent whole wheat" as shown by every nutritional experiment comparing them (52, 70, 83, 84, 107, 116).

Taylor (91) in summarizing the various methods to correct the deficiencies of white flour referred to the return to whole wheat in the following manner, "Revival of old-fashioned graham flour, despite its instability, dark color, bitter taste, high roughage and inadaptability to many commercial and household uses." This characterization of whole wheat flour is unfair. Whole wheat flour does not have a bitter taste unless it is rancid. Its instability is a commercial problem which has proved no obstacle in the past and certainly should

be less of one now. As for the dark color of whole wheat bread, that will progressively become less of a hindrance towards its acceptance as people learn to appreciate that some vitamins are pigments and the dark color may become one of the commercial advantages of whole wheat bread. Otherwise, Taylor (91) has given us an excellent discussion of the background of the nutritional problem involved in fortification under the title "Why enrichment of flour."

THE ARGUMENT OF PALATABILITY. The unpalatability of whole wheat bread and the refusal of people to eat it, even if it is to their advantage, forms the foundation of the argument for fortifying white flour. There is a fatalistic conviction on the part of the proponents of fortification that the general consumption of whole wheat bread is a thing of the past and as beyond recall as split milk (92, 93, 94, 95, 19).

Much has been made of the meager results attending the efforts of nutritionists to get people to eat whole wheat bread (93, 94, 95). It should be kept in mind that nutritionists have not until recently recommended whole wheat bread except in an academic way because they were under the influence of the doctrine of "protective foods" and whole wheat was not considered a "protective food." The term "protective food" was coined by McCollum and originally included only milk and green vegetables (96). The term was later extended to include fruits and eggs and in 1936 it was proposed to include meat also (96). Whole grain cereals were only then "nominated" for inclusion in the term "protective foods." The nutritionists taught "that in obtaining an adequate diet one must be sure to get the necessary vitamins and minerals first from other sources" (95), namely, the "protective foods," and then complete the diet with any food to suit the appetite such as white flour and potatoes. Sebrell (95) pointed out that this resulted in a decreased consumption of flour, since the appetite was satisfied to such a large extent by "protective foods." It now appears that whole wheat bread because of its cheapness and abundance is one of our most important "protective foods," but the knowledge that whole wheat is a "protective food" is so recent as to have had no great effect upon its advocacy by nutritionists.

Much is made of an attempt on the part of the Swiss Government to induce people to eat whole wheat and undermilled flours (97). In order to prevent a rise in the cost of living, the Federal Council of Switzerland put on the market a brown bread consisting of four-fifths wheat flour and one-fifth rye flour, the degree of extraction being fixed at 82 to 85 per cent. This bread sold at a somewhat cheaper rate than ordinary bread. Although no special propaganda was made for the bread, the consumption reached a level of 65 per cent of the total bread produced the first month, falling to 16.5 per cent nine months later, and finally after a year dropped to 11 per cent of the total bread consumption. "The new cheap brown bread had, in fact, only replaced the other brown breads previously eaten and had not permanently replaced white bread to any appreciable extent."

This experience has been overemphasized as an argument showing that people will not eat whole wheat bread. The report consisted of verbal information from Dr. O. Stiner, Federal Service of Health, Berne. There is nothing in the

report to suggest whether it was the consumer or some difficulty in the nature of the flour or its treatment during milling or baking which was responsible for the decrease in consumption of the special bread. Furthermore, an active educational campaign should always accompany a step of this kind.

While insisting that people won't eat whole wheat bread, the proponents of "enrichment" of white flour ignore accumulating evidence that people are slowly but surely turning to whole wheat bread. They ignore in the first place that hundreds of millions of people are eating whole wheat bread and are liking it. The American people are no different as human beings from the European peoples.

In Aberdeen school canteens (98) experiments were conducted with the object of educating children to eat more wholesome food. While difficulties were experienced with some types of food, none was experienced with whole wheat bread, showing that it was palatable to these children.

In a study of actual foodstuffs consumed in various cities in Great Britain, Cathcart and Murray (99) had this to say about St. Andrews, "The 'Bread' group provides two interesting items of information. The level of consumption of brown bread might almost be regarded as an index of social standing. Group I consisted of the highest income group. They all ate brown bread. As income progressively decreased, brown bread consumption decreased. Of the families in the lowest income group including the unemployed, only 1.3–8 per cent ate brown bread." Presumably the higher income groups consumed brown bread because they had been educated to its superior nutritive value, and it must have been palatable or they would not have eaten it. Some white bread was eaten in all classes.

In a survey in Glasgow (99) in 1911 white bread was apparently universally eaten, whereas in 1933 about one-fourth of the families ate brown bread.

It appears therefore that there are other reasons besides palatability for the consumption of white bread or whole wheat bread. When people are convinced whole wheat bread is superior to white bread, they find it palatable and when the two breads are compared, many find whole wheat bread more palatable than white bread. The unpalatability of whole wheat bread and the alleged refusal of people to eat it are myths and have no foundation in fact.

The "enrichment" of bread is frequently compared with the fortification of milk with vitamin D. That comparison does not stand scrutiny. Milk is a natural product and is practically a complete food, but it is low in vitamin D. Addition of vitamin D improves it beyond its nutritional value as a natural product, especially for children. Wheat, on the other hand, is first processed and converted from a food of high nutritive quality to a food of very low quality. To this nutritionally impoverished product, thiamine, riboflavin and nicotinic acid are added and the product is called "enriched." Actually, compared to whole wheat it is still nutritionally impoverished, and the term "enriched" bread is very misleading.

In their recommendations of daily allowances for specific nutrients (117) the Committee on Food and Nutrition fails to point out that these allowances are applicable only when natural foodstuffs comprise the diet. Then it can be

safely assumed that the unlisted members of the vitamin B complex as well as other factors are also furnished in adequate amounts. That assumption cannot be made when "enriched" bread forms a substantial part of the diet, since on paper such a diet will meet the recommended daily allowances of those specific nutrients mentioned, but will fail to supply a great many other nutrients not listed which may well be as important as those that are listed.

OPPOSITION TO ENRICHMENT. To this program for "enriched" bread, there has been no great opposition recorded in the literature, but apparently there has been a great deal of opposition which has not come out in the open as shown by the statement of Williams (94), "Yet this great reform is being sabotaged or damned with faint praise by half the nutritionists of the country on the ground that it would be still better if we could arrange breakfasts of ham and eggs, whole wheat buns and a glass of milk for everybody. Of course, it would be, but shall we wait for the millenium to take our first steps to mass repair our nutritional errors."

Luck (103) suggested that white bread be taxed as a luxury and the money thus obtained be used to subsidize whole wheat bread by establishing a price differential in its favor. This suggestion was coldly received or entirely ignored.

Carlson (104) contributed the following sound nutritional advice, "Nutritional safety lies in omnivorousness, in consuming, so far as possible, foods in their natural states, and, in the case of fruits and vegetables, eating some of them raw. Some of our malnutrition started with the processing, the refining, and the purification of such foods as the cereal grains, modern milling processes shunting the most valuable part of these natural foods into the mouths of chickens, cattle and hogs. The germ and the outer coats of the grains hold valuable proteins, minerals and vitamins. Human dietary safety on this front would seem to be: Go back to first principles; putting the whole grain into the flour and the bread. We can learn to like it. There is no more purity or virtue in white bread than in white winter butter. I think we could learn to prevent the oxidative rancidity of the whole grain. And until we have that problem licked, why not store the wheat and mill the flour as we need it.—In my judgment, the recent addition of a little of the vitamins and minerals now milled out of the grain, and singing paeans of dietary salvation over this 'enriched' flour and bread, is not a sound policy either for today or tomorrow." In fact, Carlson has been the only American scientist of note who has so far come out squarely for whole wheat flour as against "enriched" flour.

FURTHER EXPERIMENTAL WORK. *Cereals and calcium metabolism.* Evidence is rapidly accumulating to indicate there is a factor or factors in cereals which interfere with calcium metabolism. E. Mellanby (121) showed that on diets without vitamin D, rickets in puppies tended to be more severe on cereal than on non-cereal diets. He (122) later showed that under the conditions of his experiments, oats was the most rachitogenic of the cereals investigated and white flour the least, with but little difference between white and whole wheat flour. He demonstrated that vitamin D and calcium salts could counteract the rachitogenic activity of the cereals, but noted that inorganic phosphates were without

effect. Since calcium salts could overcome the rachitogenic activity of cereals, he found it necessary to explain why the cereals like oats with a high calcium content were more rachitogenic than white flour with a low calcium content. This he did by assuming that cereals contained a "toxamin" which rendered calcium salts unavailable. He found that boiling with 1.0 per cent hydrochloric acid destroyed this "toxamin," making the calcium of the cereal available for metabolic purposes. This "toxamin" could also be rendered innocuous by "saturation" with calcium salts, after which dietary calcium would be available for metabolic purposes.

May Mellanby (123) confirmed the destruction of the anticalcifying principle of cereals by boiling with 1.0 per cent hydrochloric acid. She also studied the effect of germination on the anticalcifying principle of cereals and obtained irregular results. She suggested that when loss of anticalcifying power did occur in cereals, it was due to enzymic changes.

The possibility that the phytin of cereals might be the offending substance was entertained as a result of the investigations of Steenbock, Black and Thomas (124), Templin and Steenbock (125), and Bruce and Callow (126). These workers showed with rats that rachitogenic diets with a high calcium-low phosphorus ratio could be corrected by the addition of inorganic phosphates. Part of the rachitogenic effect of the diet was due to the unavailabity of the phosphorus in the phytin of the cereals. Lowe and Steenbock (127) further showed that the availability of phytin-phosphorus depended on the conditions of the experiment. On low calcium-low phosphorus diets, phytin could be hydrolysed in the gastrointestinal tract of the rat to a substantial though incomplete degree. Calcium carbonate inhibited the hydrolysis of the phytin. They established the absence of phytase, the phytin-hydrolysing enzyme, in the intestinal mucosa of the rat and suggested that the phytase activity in the rat was probably due to the phytase of the flora of the gastrointestinal tract as well as the phytase of the ingested food.

Harrison and Mellanby (128) insisted that the findings with rats on rachitogenic diets with a high calcium-low phosphorus ratio had no bearing on his studies with puppies, since inorganic phosphates could not counteract the rachitogenic action of the cereal diet, but calcium salts could. However, they investigated the possibility that phytin might interfere with calcium metabolism. They found commercial phytin slightly antirachitic, but sodium phytate was definitely rachitogenic. They could account for the rachitogenic action of cereals by their phytin content. The phytin from cereals contained less calcium than commercial phytin. The high calcium of commercial phytin could account for its slight antirachitic activity. They (128) concluded "This view that the action of cereals is due to interference with calcium absorption accords with the observation that the effect can be prevented by feeding extra calcium, in other words, by saturating phytic acid and rendering it inactive." After saturating the phytic acid with calcium, further additions of calcium would presumably be available to the animal.

While Harrison and Mellanby (128) had established that the phytin in cereals

was, at least in part, the offending rachitogenic substance, their contention that phytin rendered calcium unavailable by combining with it to form an insoluble calcium phytate was put into serious doubt when Krieger et al. (129) showed that the calcium of calcium phytate was as readily absorbed as that of calcium carbonate when fed to rats on cereal-free diets low in calcium. There remained to be explained the mechanism by which phytin interfered with absorption of calcium.

McCance and Widdowson (130) made a thorough investigation with human beings of the absorption of calcium from diets in which either brown bread (made from flour of 92 per cent extraction) or white bread made up 40 to 50 per cent of the calories. More calcium was absorbed from the white bread diet even though the calcium intake was less than that on the brown bread diet. Fortifying the breads with calcium salts increased the absorption of calcium and prevented loss from the body if it had been taking place. The carbonate was as effective as the phosphate. Vitamin D did not improve the absorption of calcium in the human adult. Sodium phytate when added to the white flour diet interfered with the absorption of calcium. Yet, sodium phytate was split in the gastrointestinal tract since about one half of its phosphorus was absorbed (130). How phytin could be split to such a large extent and still interfere with calcium absorption was a puzzle. It was explained on the assumption that calcium was absorbed in the upper part of the gastrointestinal tract, while phytin was split in the lower, and any unavailable calcium liberated in the splitting of the phytin was lost with the feces. These investigators (130) showed that dephytinized bread lost part of its power to interfere with calcium metabolism, while phosphates were shown to account for the remainder of the anticalcifying action of brown bread. The bulk of the feces had little to do with the absorption of calcium. They attributed the poor absorption of calcium from brown bread to the specific action of the phytates and phosphates. The dephytinization of bread deserves greater study. The great variability among their subjects in their ability to absorb calcium was one of the outstanding findings of their study.

On the basis of their studies (130) they recommended that flours be fortified with calcium as follows: For each 100 grams of white, 85 per cent and 92 per cent flour, they recommended the addition of 65 mgm., 120 mgm. and 200 mgm. respectively of calcium. When brown bread forms about 40 to 50 per cent of the caloric intake, a little less than a pint of milk would supply adequate calcium in the absence of other rich dietary sources of calcium.

The problem of calcium metabolism is a complicated one. The normal man secretes 4 to 10 liters of digestive juices containing 0.3 to 0.8 gram of calcium (131). Different types of food cause the secretion of different amounts of digestive juices. Whole wheat bread will stimulate the secretion of a greater quantity of digestive juices than white bread (35). This calcium must be reabsorbed or lost to the body. The absorption of calcium is influenced by phytates, phosphates, vitamin D, fat, citrates, oxalic acid, acid: base balance and many other factors (131).

Cereals and iron metabolism. Widdowson and McCance (132) cautioned

against laying too much stress upon brown bread as a food rich in iron. This recommendation was based on some balance studies on white and brown bread with normal men and women. More iron was retained on white bread than on brown, even though the latter bread contained more iron. Positive balances were, however, obtained in all cases. Data on balance studies, especially when positive balances are obtained, are hardly grounds for condemning the biological value of iron in whole wheat in the face of such overwhelming data in the rat indicating that the iron of whole wheat has a very high biological value as measured by hemoglobin formation (138, 107), a far more pertinent criterion than a small or large positive balance. In this respect the work of Mitchell et al. (107) is especially significant. They showed with rats that a whole wheat diet induced a significantly higher hemoglobin production than an "enriched" white bread diet, even though the "enriched" white bread diet contained a little more iron. Widdowson and McCance (132) cautioned against applying the results of experiments with rats to man because the rat secretes a phytase (133) and man presumably doesn't. Therefore phytin would presumably interfere with iron metabolism in man but would do so to a lesser extent in the rat. This criticism is, however, not valid. There is no agreement on the ability of the rat to secrete a phytase since Lowe and Steenbock (127) could not find any phytase in the intestinal mucosa of the rat but attributed the phytase activity in the rat to the flora and the phytase of the food. Moreover, the human in this respect does not differ so markedly from the rat, since there must be a good deal of phytase in the human intestinal tract to account for the splitting of about half of the ingested phytin (130).

Studies on iron absorption seem difficult to interpret. Widdowson and McCance (132) suggested that little iron is excreted in the gut except through bleeding and iron is poorly absorbed except when needed by the body. These ideas have been amply confirmed (134). Patients bleeding from peptic ulcers absorbed 15 to 20 per cent of the iron administered whereas a normal medical student absorbed only 1.8 per cent. Patients with hypochromic anemia absorbed a great deal more iron than normal people. In general, it seems that the question of dietary iron has assumed an importance unjustified by the probable rôle it plays in the diet of the population (135).

Feeding experiments with whole wheat and "enriched" bread. One of the most exhaustive and best planned investigations of the nutritional value of "enriched" white flour is that of Mitchell, Hamilton and Shields (107). Three series of experiments were run. The first series compared "enriched" white flour, "enriched" white flour with 6 per cent skimmed milk solids, and white flour with milk solids. The paired feeding method was used with equalization of food intake. The rats were paired in trios. The test showed that the flours containing the milk solids were biologically equal whether enriched or not, and superior to the "enriched" flour without the milk solids. "Enriching" flour containing 6 per cent milk solids added nothing not already added by the milk.

In the second series of experiments, paired trios were again used comparing whole wheat flour, "enriched" white flour without milk solids, and "enriched"

white flour with milk solids. Again, the "enriched" white flour was inferior to the "enriched" white flour plus milk solids and also to whole wheat flour. There was no significant difference in nutritive value between whole wheat flour and "enriched" white flour plus milk solids.

In a third series of experiments, growth instead of food intake was equalized between the trios, thus comparing the nutritive value of the different flours in terms of the amount of food required by the rat to make the same growth. In this series they compared whole wheat bread, whole wheat bread with milk solids, and white bread with milk solids. The test showed that whole wheat bread was somewhat inferior nutritionally to white bread plus milk solids and whole wheat bread plus milk solids was superior to the other two breads. It required 11 per cent less food as whole wheat bread plus 6 per cent milk solids than white bread plus milk solids for rats to grow at the same rate.

Whole wheat bread gave somewhat better iron retention and hemoglobin formation than breads "enriched" with iron salts, with or without milk solids. On the other hand, whole wheat impaired somewhat the utilization of calcium.

Williams, Mason and Wilder (106) have evaluated the nutritive contribution of "enriched" white flour "with particular reference to satisfaction of the human requirements for thiamine and riboflavin." Seven subjects, all women, were used in this test and divided into 3 groups. White flour, white flour enriched with thiamine, nicotinic acid and 6 per cent milk solids and whole wheat flour were compared. The flour made up about 30 per cent of the calories of the diet, the rest consisting of foods commonly appearing on American tables. The experiment was badly planned since neither the whole wheat flour nor the unfortified white flour contained milk solids, though it is a common practice of commercial bakers to add milk solids to white bread. Properly planned, the experiment should have compared either white flour and "enriched" white flour or white flour with milk solids and "enriched" white flour and milk solids. The omission of milk solids from whole wheat flour was perhaps justifiable since commercial whole wheat bread does not usually contain milk solids. The addition of milk solids to commercial whole wheat bread should be encouraged.

The subjects on the white flour diet developed symptoms of thiamine deficiency, but so did those on the "enriched" flour, though only in a mild form. One subject on the whole wheat flour developed symptoms of thiamine deficiency and one was symptom free. There were no definite symptoms of riboflavin deficiency in any of the subjects. Their results could not be confirmed by Keys et al. (118) who fed young men an equally low thiamine diet and subjected them to hard work. No interference in carbohydrate metabolism nor any other symptoms of thiamine deficiency were noted.

The vitamin B-complex and hard labor. Johnson et al. (108) investigated the effects of a diet, deficient in the vitamin B complex upon men doing manual labor. They showed that men doing hard physical labor even for a few days need an adequate intake of the vitamin B complex. Thiamine alone will not maintain the physical fitness of laborers, but whole dried brewer's yeast will. This work has been confirmed in part by Barborka, Foltz and Ivy (109) who

showed that the vitamin B complex is necessary for hard physical labor. These workers used a yeast concentrate as their source of vitamin B complex.

The inability of thiamine to restore the physical fitness of men, deficient in the vitamin B complex, for doing hard physical work shows the danger of jumping to conclusions about the importance of thiamine in the human dietary. The rôle of thiamine in carbohydrate metabolism has led to the overemphasis in its importance in activities involving accelerated oxidations. To be sure, thiamine is necessary, but so are other factors and an adequate diet may be compared with a chain whose strength is determined by the weakest link. In the same way a diet becomes deficient if it is deficient in any single factor. To replace whole wheat, whose nutritional quality is known, by an unknown quantity like "enriched" white flour is unwise. In this connection it should be pointed out that eight synthetic vitamins were inadequate as a source of the vitamin B complex for the monkey (119) and for the puppy (120). Should the intake of "protective foods" in our population be reduced below a safe level, the result may be nutritional disaster. This crisis is no time to play a guessing game with the nutritional welfare of 130,000,000 people at stake, especially when all evidence points to the nutritional superiority of whole wheat bread. The argument of palatability is dubious ground for such a course.

COMPOSITION OF WHITE FLOUR, "ENRICHED" WHITE FLOUR AND WHOLE WHEAT FLOUR. While the nutritional superiority of whole wheat over white flour has been established for a long time by many feeding experiments, it is only recently that the reasons for this have been understood. As new factors of biological importance have been discovered, whole wheat and more especially the germ and bran were found to be good sources of these factors. In table 8 white flour, "enriched" white flour and whole wheat flour are compared with respect to all nutrients for which data could be found. The superiority of the whole wheat flour is impressive and explains the superiority found for whole wheat in feeding experiments. It should be noted that the table is incomplete, since other factors such as biotin, choline, inositol and folic acid are not listed for lack of data and they are, so far as known, also concentrated in the wheat bran and germ. Other less well known factors, and perhaps factors as yet undiscovered, are also likely to be present in these mill by-products.

It is difficult to see how in the face of this impressive evidence of the nutritional superiority of whole wheat bread, backed as it is by so many feeding tests, that the Committee on Food and Nutrition could recommend "enriched" white flour, at a time when "protective foods" of animal origin are becoming scarcer and are bound to become increasingly so under our war economy. They have recommended that we throw away our "staff of life" upon which we may have to lean very heavily and substitute in its place a crutch of known inferiority, of uncertain quality and untested value. It is altogether possible with our rapidly deteriorating food situation, if the war continues for any great length of time, *and especially in event of poor crops*, that we shall have to depend upon bread for a larger share of our food. Then the wide use of "enriched" bread might result in an impairment of the nutrition of large numbers of our people.

We do not know enough about food deficiencies in the population. The clinical information is spotty and unreliable, since no reliable criteria exist to characterize these vague subclinical nutritional deficiencies. The claims made for nutritional deficiencies in the population are based largely on dietary standards of various food factors and also nutrition surveys both of which are unreliable. The dietary standards are little more than intelligent guesses at human requirements, and care has been taken to set them high enough to allow for margins of safety which is all to the good, but partial deficiencies of specific members of the vitamin B complex in the general population cannot at present be determined with any degree of certainty.

TABLE 8

Composition of white flour, "enriched" white flour and whole wheat flour

	WHITE FLOUR	"ENRICHED" WHITE FLOUR	WHOLE WHEAT FLOUR	REFERENCE
Thiamine, mgm. per pound	0.3	1.7	2.3	(110)
Riboflavine, mgm. per pound	0.15	1.2	0.6	(110)
Nicotinic acid, mgm. per pound	3.5	6.0	26.0	(110)
Pyridoxine, mgm. per pound	1.0	1.0	2.0	(101)
Pantothenic acid, mgm. per 100 grams	2.5	2.5	5.0	(101)
Carotene (vitamin A), mgm. per pound	nil	nil	1.5	(111)
α-Tocopherol (vitamin E), mgm. per pound	nil	nil	1.4	(112)
Fat, per cent	1.2	1.2	2.4	(111)
Protein, per cent	11.0*	11.0*	12.7†	(111)
Calcium, per cent	0.02	0.02	0.045	(111)
Phosphorus, per cent	0.092	0.092	0.423	(111)
Iron, mgm. per pound	3.0	6.0	20.0	(111)
Manganese, grams per 750 calories	0.1	0.1	6.7	(86)
Potassium, per cent	0.115	0.115	0.473	(111)
Copper, grams per 750 calories	0.40	0.40	1.6	(86)
Ash, per cent	0.37	0.37	1.70	(111)

* Low quality.
† High quality.

The proponents of enriched bread are on tenuous grounds when they claim:

1. The major vitamin B complex defects of the American diet are thiamine and nicotinic acid.

2. That these are the only valuable factors of the vitamin B complex supplied by whole wheat.

3. That the rest of the diet contains an abundance of the other vitamin B complex factors (plus factors) of whole wheat, thus justifying their removal in the milling process.

The plain truth of the matter is that we do not know. It would be advantageous to use whole wheat bread for the following reasons:

1. The vitamin B complex with the exception of riboflavin would automatically be taken care of. Losses in cooking would not have such a serious effect on the diet.

2. Unknown factors would be supplied giving greater confidence to the diet, since some of them may not be adequately furnished by the rest of the diet.

3. Proteins of high quality and minerals would be furnished.

4. Milk would supplement the wheat by making good the riboflavin deficiency, improve the quality of protein and add enough calcium to make it adequate. Milk production is being encouraged at the expense of meat production.

5. With milk and whole wheat bread the diet would practically be adequate and addition of small amounts of eggs, fish, fruits and vegetables would round out the diet and make it nutritionally excellent.

6. It is always far easier to build an adequate diet around whole wheat flour than white flour.

7. As foodstuffs such as meat, eggs, milk, fruits and vegetables become scarce as a result of the war, the whole wheat, if used, will become the mainstay of our diet and if properly combined with what protective foods will be available, the nutritional level of the population will remain high. If white flour is used, even if "enriched," it is difficult to see how deterioration of the national diet can be avoided. That would indeed be a matter of grave concern.

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THE CHANGES IN THE FETAL CIRCULATION AT BIRTH

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The pattern of the fetal circulation and the changes it undergoes when breathing begins at birth have long been topics of interest and speculation, but seldom subjects of direct observation. As a result the greater part of our knowledge about them was, until recently, inferred from observations made post mortem upon fetuses at verying stages during gestation and upon the new born. Within the past ten years, however, a great deal of information has been accumulated by direct methods in studies carried out in, and inspired from, the laboratories of Sir Joseph Barcroft at Cambridge and A. E. Barclay at Oxford.

As is often the case the advances in our knowledge have resulted from the application of new techniques to the age old problems. Barcroft has studied intensively the oxygen content of the fetal blood to discover the pattern of the circulation. Barclay has brought to a high degree of perfection methods for making x-ray cinematographs. These methods have enabled him and his collaborators to record pictorially the movement of radiopaque substances such as thorotrast, uroselectan B, and lipiodol, after their introduction into the blood stream, and so the movement of the blood. The records are of two types: direct and indirect. The direct records are made by exposing five inch segments of a continuous film five inches wide and many feet in length. Such exposures have been successfully made at a rate of three or four per second. Indirect records are made by photographing with a sixteen millimeter cine camera, at normal speed, the images made by the x-rays on a flourescent screen.

The application of these new techniques has clarified our views, but even they do not permit the study of the circulatory changes *in utero* and at normal birth. To apply them the fetus must be delivered by caesarian section. Under the most favorable conditions the uterus after delivery begins to contract and impair the circulation through the fetal and maternal sides of the placenta. Until someone devises methods for study of the fetal circulation *in utero* we shall be obliged to deduce its character from fetuses under such conditions. Despite this limitation the available data do serve to broaden our view about the nature of the vascular system in prenatal development and at birth. To this end they are collected here.

The pattern of the fetal circulation immediately after delivery by caesarian section, placental circulation intact. Radiopaque substances introduced into the umbilical veins of near term fetal lambs (27) are carried to the posterior vena cava along either one of two routes. One leads from the umbilical recess—the proximal portion of the vein formed by the union of the two umbilicals—through the liver capillaries. From these capillaries the blood is collected by two large hepatic veins and a varying number of small vessels all of which empty into the posterior cava. The second route leads from the umbilical recess through the ductus venosus—thus avoiding the liver capillaries—into the vena cava.

There has been speculation from time to time as to the portion of the return from the umbilical veins that follows each of these routes. The films of Franklin, Barclay and Prichard (27) demonstrate that in the sheep the greater part goes through the liver capillaries; the smaller through the ductus venosus. This evidence is in accord with the older anatomical studies which indicated that the ductus venosus in the human fetus at term had a diameter $\frac{1}{7}$ of that of the umbilical vein (54). The portion of the blood traversing the ductus may—under circumstances not well understood—be diverted into the liver capillaries.

The x-ray records of fetal lambs reveal that the flow through the ductus may cease abruptly only to begin again after intervals of varying length. The cessation and renewal of the flow through the ductus venosus resembles in some respects the arrest of the blood flow through the ductus arteriosus at birth (see below). It appears to be brought about, as Barclay and his colleagues suggested (8), by the contraction of a horseshoe shaped sphincter muscle in the wall of the umbilical recess, just at the point where the ductus springs from it (20). This muscle is supplied, in the sheep, with motor type endings by a nerve formed by union of branches of the two vagi. These nerve fibers do not appear to end on ganglion cells in or near the umbilical recess but directly in its musculature. Hence they are probably not parasympathetic preganglionic but post ganglionic sympathetic fibers. This assumption is supported by the observation of Franklin (personal communication) that stimulation of the vagi in the neck region of fetal lambs was not followed by constriction of the sphincter in a single experiment. Further observations will be necessary before any final statement can be made.

Studies of the activity of this sphincter as revealed by x-ray cine records have been made only in fetuses near term. It may be that the sphincter is active only in the closure of the ductus at birth. However, the peripheral mechanism is laid down and anatomically complete by the fortieth day of gestation (20) so that it may play a rôle in regulating blood flow through the liver in normal uterine life. If active, it might be expected, by constricting, to raise the blood pressure in the umbilical veins and to lower it in the cava. As will be pointed out below, lowering the pressure in the posterior cava might have important consequences at the heart.

To return to the course of the blood leaving the liver: In the posterior vena cava the blood bearing the contrast material is added to the return from the caudal half of the body. Upon its approach to the heart, as indicated by the x-ray records, this stream divides (5). The deeper shadow indicating the larger stream of blood enters the left heart via the foramen ovale; the smaller stream, casting a shadow less dense enters the right atrium.

In the left heart the stream from the posterior cava is added to the return from the lung capillaries and projected through the aortic arch into the head, the upper extremities and the dorsal aorta. The stream into the right heart is added to the entire return from the upper circulation arriving via the anterior vena cava, for the radiographic studies indicate that none of the anterior caval flow enters the left heart directly. The contents of the right heart are projected—as indicated

by the shadows cast by contrast materials borne in them—along the pulmonary aorta, to the lung capillaries and through the ductus arteriosus into the dorsal aorta. The blood in the dorsal aorta represents contributions from both ventricles. This pattern of the fetal circulation appears to be substantially the same through the last third of the gestation of the fetal lamb. It can be represented diagrammatically as in figure 1.

Similar results with regard to the course of the two caval streams into and through the heart have been obtained in kitten and guinea pig fetuses by Windle and Becker (51). Their methods were simple and direct. In these fetal types,

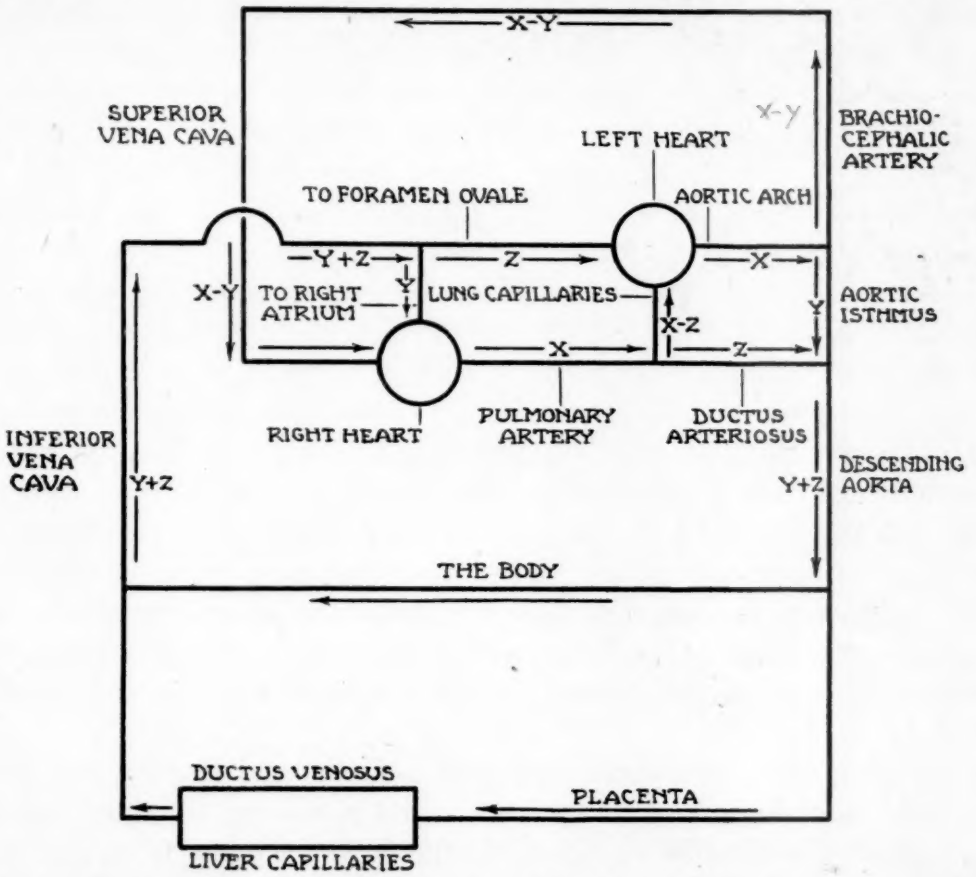

Fig. 1. A scheme representing the basic plan of the fetal circulation. The arrows indicate the direction of blood flow. The blood flow per minute in each of the major channels is expressed algebraically. For details see text.

if the chest plate is removed, the atrial walls are thin enough to permit the detection of small differences in the color of their contents by inspection. India ink in small quantities was introduced into either the umbilical or jugular veins. Ink introduced into the umbilical vein passed for the most part directly into left atrium as indicated by its darkening; a small quantity of ink entered the right atrium. Ink injected into jugular vein did not darken the left atrium on reaching the heart, but passed through the right atrium and ventricle to the ductus arteriosus and the lungs.

Some of the consequences of this pattern of circulation have been revealed by a study of the oxygen content of the blood in the umbilical vein and arteries,

and in the carotid (30, 13). In the fetal lamb the amount of oxygen in the carotid blood is intermediate between that in the dorsal aorta—as represented by the umbilical artery—and that in the umbilical vein. Until a few days before birth it is always nearer the value for the umbilical artery than that for the vein; but in the last days of gestation the oxygen content of the blood in the carotid tends to drop to a value nearer that of the umbilical artery. Substantially the same results were obtained by Huggett (30) on goat fetuses, though his series is not so complete nor does it include observation on fetuses in the very last days of gestation.

These three lines of evidence indicate that the blood returning from the placenta rich in oxygen is distributed in the main to the upper circulation, which includes the heart itself, the upper extremities, the central nervous system and a part of the chest wall. But the significance of this distribution remains to be discovered.

The anatomical basis of the pattern of the fetal circulation. The establishment of the pattern of the circulation through the fetal heart raises the question: How is this selective distribution of the two caval streams accomplished? Apparently the failure of any one in the past to demonstrate to general satisfaction any mechanism responsible for a selective distribution of the two streams in the heart has proven the greatest obstacle to a widespread acceptance of the Sabatier (47) theory. Sabatier believed that all of the blood reaching the heart from the inferior vena cava passed directly into the left atrium via the foramen ovale without entering the right atrium. From the left atrium it was distributed to the head and upper extremities. The blood returning to the heart from the superior vena cava, he believed, passed through the ductus arteriosus into the descending aorta thence to the lower parts of the body and the placenta. None—according to the hypothesis—passed through the lungs; none through the isthmus of the aorta.

The opposing view, erroneously credited to Galen and Harvey (see Franklin, 26) assumed that the two caval streams mixed in the right atrium and the left atrium was supposed to be supplied via the foramen ovale from this mixture. Both ventricles pumped blood of the same composition into their respective beds. The circulation through the lungs was assumed to be minimal. This view has been supported, to be sure, by anatomical and physiological studies (43, 44, 32, 33, 39, 40) but its popularity appears to have stemmed in a great measure from its inherent probability. This inherent probability, of course, rests on the assumption that the two cavae end in the right atrium and only there, and further that the foramen ovale is the only direct communication between the two atria. These assumptions—a part of current teaching of the embryology of the heart—have had their contestants along the years, chief among them Wolff (53), Kilian (36), Rudinger (46), Zeigenspeck (54) and recently Amoroso *et al.* (2).

Wolff was apparently the first to advance, on the basis of anatomical studies made upon human fetuses, the view that the atria in the fetal heart are not in direct communication with one another, but are joined through the channel of the inferior vena cava which opens separately into each chamber. Wolff's con-

tention was supported later by the anatomical studies of Kilian (36) and of Zeigenspeck (54). In his descriptions of the termination of the cavae in Preyer's book, *Die Physiologie des Embryos* (45), and in his later paper (54) Zeigenspeck provided illustrations and diagrams that would appear to establish Wolff's view as a fact, but these were either ignored or did not come to general attention.

Lately the subject has been restudied by Amoroso *et al.* (2), who pointed out that in the light of recent embryological studies a part of Wolff's inferior vena cava would not be so described now, for it arises from cardiac tissue. However, any difficulties that might arise from the use of anatomical terms with fixed implications may be avoided by the use of their term "posterior caval channel" for designating the structure as it appears in the fetal lamb. Under this term Amoroso and his colleagues would include not only the posterior cava itself,

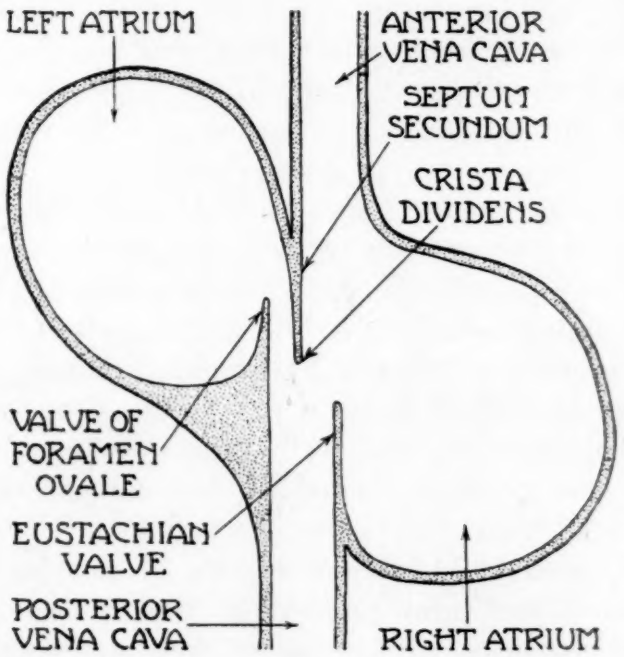

Fig. 2. A diagram illustrating the relations of the termination of the posterior caval channel and the interatrial septum in a mid-term fetal lamb.

but also the intracardiac continuation of the channel up to the level at which the posterior caval blood discharges into the atrial cavities. In a study which included a variety of forms—ungulates, carnivores and primates—but gave special attention to the lamb, Amoroso *et al.* found that the division of the posterior caval channel into the right and left branches occurs on the free edge of what embryologists in this country would term the septum secundum or isthmus atriorum. They purpose the term "Crista dividens" to designate this edge—a term surely more indicative of its functional rôle but one which gives little hint of its embryological origin. In the fetal lamb at term (see fig. 2) the left boundary of the left fork of the posterior caval channel is the valve of the foramen ovale or septum primum; the right boundary of the right fork of the channel opening into that atrium is formed by the Eustachian valve or the right valve of the

sinus venosus. The left fork of the post caval channel leads, therefore, directly into the left atrium, the right into the right atrium. According to Amoroso *et al.* (2) this general plan is found in the other forms they studied.

This view of the configuration of the termination of the inferior vena cava and the position of the foramen ovale has been repeatedly opposed (21, 44), especially by Born who objected that the relationships of the parts could not be established on fresh specimens in which they could be pushed and pulled about; an objection that seems to have been regarded as valid by the vast majority of embryologists for Wolff's interpretation does not appear to have been generally accepted.

Further anatomical studies will doubtless be needed before Wolff's views become a part of current teaching, but the fact remains that they do account for the results which have been obtained from the studies on the course of the posterior caval blood through the heart. These studies in turn are a confirmation of his scheme of the fetal circulation, for Wolff expressed the opinion about one hundred and sixty years ago that the posterior caval blood was poured into both atria—one-third of it into the right, two-thirds into the left. After one hundred and sixty years these views may be regarded as substantially correct for the final stages of development.

One further anatomical point merits attention; how is the anterior caval blood prevented in the fetus from entering the posterior caval channel and so entering the left heart? This separation appears to be accomplished by a tubercle—the tubercle of Lower—interposed between the central ends of the two cavae. According to the description of it given by Franklin, Barclay and Prichard (27), this tubercle in the fetal sheep forms a part of the dorso-anterior wall of the posterior cava and the postero-dorsal wall of the anterior. Its rôle in the separation of the caval streams they have deduced by a comparison of radiographs of the roots of the great veins with dissections of them.

The structure of the heart and the roots of the great veins appears, therefore, to be compatible with, and in all probability responsible for the course of the blood into the two ventricles. If the rôle of these structures in the functional separation of streams to the right and left hearts needs further emphasis it has been provided in a striking manner by Whitehead's (50) neoprene model—a model based upon a reconstruction from serial sections, of the right atrium, the caval terminations, the foramen ovale and the right atrioventricular opening of a fetal kitten killed *in utero*. When perfused via the "inferior vena cava" with red colored water at 75 mm. Hg and at the same time through the "superior cava" at 67 mm. Hg with blue, the fluid which emerged from the "foramen ovale" contained 92 per cent red from the "inferior cava" and 8 per cent blue from the "superior". The fluid leaving the "atrioventricular orifice" was 87 per cent blue from the "superior cava" and 13 per cent red from the "inferior". The position of the model in space had no effect upon the results. However, when the pressures were lowered and equal the two "caval" streams mixed completely before emerging at the "foramen ovale" and the "atrioventricular opening".

The rôle of dynamic factors in the maintenance of the fetal circulation pattern. There are suggestions, though no direct evidence, that in the living heart, as in

Whitehead's model, the pressure in the posterior cava must exceed that in the anterior if the normal distribution of the streams to the atria is to take place. For example, Windle and Becker (51) noticed that the caval streams underwent rather thorough mixing in the atria of kitten and guinea-pig fetuses when the heart slowed and the blood pressure fell. A fall in the blood pressure of the fetuses they were studying may have been the underlying cause for the mixing of the caval streams in the heart as observed by Pohlman (44) in pigs and by Kellog (32) in dogs—an observation that led both investigators to assume that the two caval streams normally mixed in the right atrium. Their results really bear witness to the fact that the distribution of the caval streams observed by Barclay *et al.* (4, 5) and Windle and Becker (51) depends upon dynamic factors in addition to those of configuration.

And these dynamic factors may, in some fetal forms at least, be altering a good deal from moment to moment during the last day of gestation. During that period, as is well known, the uterine contractions that earlier occur so occasionally increase in frequency. Each uterine contraction alters the fetal blood pressure by arresting circulation through the vessels of the placenta.

In the fetus a transient rise is followed by a well defined fall which lasts throughout the contraction. During the fall in pressure the fetal heart is slowed (23). This fall in pressure, other things being equal, might be expected to be accompanied by mixing of the caval streams in the heart. Similarly the narrowing of the ductus venosus and a fall in the posterior caval pressure might be accompanied by mixing of the caval streams. *In utero* the occurrence of these events certainly could not be regarded as abnormal. Perhaps we would not be far wrong if we assume that the distribution of the caval streams, as pictured by Barclay *et al.* (4, 5) and by Windle and Becker (51), represents the initial pattern from which the transition to the adult type begins—the changes in the dynamic features as in the nature of rehearsals for the first act of the drama of birth.

Variations of the circulation within the fetal pattern. There is good evidence from the studies of Barclay *et al.* (4, 5) that the pattern of the circulation remains unaltered during the last third of gestation. But within this pattern there are possibilities for wide variation in the proportions of the blood that perfuse the individual channels. Data indicating the extent of these variations are meager indeed, but certain tentative conclusions may be drawn from them.

Four facts appear to be established about the fetal circulation: 1. That the posterior caval stream divides, a part going to each atrium. 2. That the anterior caval stream enters only the right. 3. The capacities of the two ventricles are equal (44) as are—4—the pressures in them (29). Taken together the last two statements indicate that the ventricular outputs are equivalent. These facts may be related to the scheme of the fetal circulation as illustrated in figure 1. In this scheme the output of each ventricle may be called x. The output of the left is divided between the upper circulation and the aortic isthmus. The fraction perfusing the isthmus may be designated y; then the quantity passing through the upper circulation will be $x - y$. This quantity $x - y$ is returned to the right heart. To provide the output x, the quantity y must be furnished by the stream

entering the right heart from the inferior vena cava. The minute volume through the aortic isthmus appears therefore to be equivalent to that of the stream from the inferior cava into the right heart.

The output of the right ventricle x is divided between the lung circuit and ductus arteriosus. The minute volume through the ductus arteriosus may be designated z; that of the lung circuit then becomes $x - z$. The quantity z from the ductus is added to the y from the aortic isthmus and $y + z$ then represents the flow through the dorsal aorta to the body and placenta which returns through the posterior cava. Since the quantity y is diverted to the right heart, the quantity z must reach the left heart via the foramen ovale to be added to the stream represented by $x - z$ from the lungs. Upon this reasoning the minute volume through the ductus arteriosus appears to be equivalent to that through the foramen ovale. According to this scheme if z—the flow through the ductus arteriosus—equaled the output of the right ventricle, y would be zero. The two ventricles would then be operating as pumps in parallel and the pattern of the circulation would be similar to that postulated by Sabatier (47). On the other hand, as the value of z falls the proportion of the right ventricular output perfusing the lungs increases; when z equals zero, then all of the output of the right heart will perfuse the lungs and the flow through the foramen ovale and the ductus arteriosus will cease. The two ventricles would then be arranged to pump in series. One further generalization can be made about this scheme. Any increase in the minute volume of blood traversing the lung capillaries will be accompanied by a corresponding decrease in the minute volume through the lower circulation. Oddly enough the minute volume through the upper circulation remains unchanged.

Observations upon the relation between the quantities y and z. One of the conclusions arrived at above was that the division at the heart of the posterior caval stream into two parts was primarily a result of the configuration of the termination of the posterior caval channel. Professor Patten (private communication) has informed me that his studies of the relations of the posterior caval channel and the foramen ovale indicate that there is no pattern standard in all mammals and that the relations alter during development of an individual. The differences are largely the result of variations in the position of the septum secundum with reference to the termination of the posterior caval channel. The comments of Professor Patten recall the observations of Wolff (53) and Zeigenspeck (54). Wolff concluded from a study of the human fetal heart that the septum secundum was placed so far to the right with respect to the midline of the inferior caval channel that, until the third month of fetal life, almost all of the inferior caval blood was poured into the left atrium. As development advanced the valve of the foramen ovale grew and the septum secundum shifted relatively toward the left. Wolff estimated from the position of the septum secundum that near full term one-third of the inferior caval blood went into the right atrium.

The recent study of Keen (31) amply confirms Wolff's contention about the shift of the septum secundum and his excellent illustrations should be studied by

everyone interested in the subject. In the heart of the human fetus of 28 weeks illustrated by Keen the inferior caval channel opens almost directly into the left atrium, *i.e.*, completely to the left of the septum secundum, whereas in the human heart at birth the channel opens very much farther to the right. The septum secundum is placed very near the left wall of the inferior caval channel but still separated from it.

According to Zeigenspeck's (54) estimate—again based upon the position of the septum secundum in relation to the opening of the posterior caval channel in human fetuses near term—one half of the blood goes into the right atrium. This estimate has support from observations of Patten and Toulmin (42) who have calculated the cross sectional areas of the inferior vena cava and of the functional orifices of the foramen ovale in twenty stillborn babes. The cross sectional area of the cava they found to be 69.7 sq. mm., the orifice of the foramen ovale 32.2 sq. mm. Inasmuch as the pressures at these two points are the same, the flow to the left side of the heart at term would appear to be just about half of the inferior caval return, y then, on the basis of these studies would appear to be small relative to z early in gestation but to increase gradually until finally at term it is equal to z or nearly so.

These estimates of y furnish, when applied to the scheme in figure 1, an estimate of the quantity of blood traversing the isthmus of the aorta for these quantities appear to be equivalent. At term the ductus arteriosus and the isthmus must both be contributing about the same quantities of blood to the descending aorta. This supposition has further support from Patten and Toulmin's (42) observation that the cross sectional areas of the isthmus and of the ductus are nearly equivalent (actually 14.4 and 15.2 sq. mm. respectively). The pressures in the vessels are equal, hence the flow is in all probability the same in each.

There are other ways of estimating the proportion of the posterior caval flow following each route into the heart and it is to be hoped that they will be applied to the problem. Since lipiodol injected into the umbilical vein forms droplets that are carried in the blood, it should be possible radiographically to record, as Barclay and his colleagues have done, the results of such an injection and then to determine the number of droplets that followed each route through the heart. Other things being equal the drops should be in proportion to the quantity of blood following each path. Until some such technique is employed, probably no more definite conclusions can be drawn than the estimates arrived at above.

Observations on the relationship between the quantities z and x. The estimations made above which place y at the end of term equal or nearly so to z, imply that the quantiy $x - z$ must be nearly the equivalent of y and of z. In other words, they imply that at the end of gestation a very large proportion of the output of the right heart is perfusing the lungs; a contention advanced by Patten (39) on anatomical grounds.

This is certainly not true of sheep and goat fetuses during the first two-thirds of gestation. The studies of Huggett (30) on goats and Barcroft and his collaborators on sheep (10, 13) have shown quite conclusively that the amount of oxygen in the blood of the carotid is intermediate between the oxygen in the bloods

of the umbilical vein and artery (see fig. 3). In the sheep fetus until about 40 days before the end of term the percentage saturation of the carotid blood so nearly approaches that of the umbilical vein and differs so markedly from the blood of the umbilical artery, that after making due allowance for the contamination of the umbilical vein blood by the contents of the posterior vena cava, there can be little, if any, further reduction in the left atrium. The inference is of course that $x - z$ or the flow through the lungs is very small indeed. Therefore, z must be very nearly equal to x during this period and Barcroft's estimate (9) that the ductus arteriosus conveys about half of the cardiac output to the lower circulation cannot be far wrong.

During the last 40 days of gestation (13) the oxygen content of the carotid blood tends to approach that in the umbilical artery and is very near it in the last ten days. The oxygenated post-caval blood is apparently diluted to an ever increasing degree, during this period, by reduced blood. This of course does not prove that the mixing or dilution occurs in the left atrium, merely that the fall in the oxygen content of the carotid blood would not be incompatible with an increased return through the lungs. The mixing might occur as a result of an increased return through the body circulation into the posterior vena cava—a view favored by Barcroft *et al.* (13)—or as a result of regurgitation of the right atrial contents into the left via the posterior caval channel. For the latter two possibilities there is not a great deal of supporting evidence.

The view that the mixing does take place as a result of an increased lung flow is supported by the radiological studies of Barclay *et al.* (4, 5), for a considerable portion of the contrast material introduced into the external jugular vein finds its way into the lungs from the pulmonary aorta. (The proportion could be determined for any stage by the use of lipiodal drops as indicated above.) From these studies, too, comes the only indication we have of the circulation time through the lungs of the fetus (8). As judged from the time required for radiopaque substances to be carried through the lung capillaries from the pulmonary arteries to the left atrium, in quantities sufficient to produce a shadow on the film, the average circulation time is remarkably short, *i.e.*, 2.7 seconds, and surprisingly enough this figure does not appear to alter significantly during the last forty days of gestation. The blood in the lungs of the fetal sheep is therefore renewed about twenty times a minute; a surprisingly high rate.

The volume of the blood in the lungs of the fetal sheep during development has not been determined, hence we have no real idea of the quantity of blood involved. However, the vascular bed of the lung does not make any spectacular growth in the last ten days of gestation, during which, if born, the fetus will survive, so its size and the amount of blood in it during these days must be about equivalent to that in the newborn lamb. At least this appears to be the case in the fetal types in which the blood volume of the lung has been estimated. Abel and Windle (1) have determined the total iron content and found equivalent amounts in the lungs of full term kittens before birth and after breathing. The inference is that the amount of hemaglobin and hence the amount of blood was the same in both circumstances. In the human fetus at term the lungs have been

found to contain 5 per cent of the total blood volume (37); the normal amount in the adult lung is usually given as between 5 and 7 per cent. If any similar proportion of the total blood volume is present in the lung of the fetal lamb, during the last ten days of pregnancy, the amount circulating through the lungs must be very large indeed for the estimated blood volume of the fetus near term is between 300 and 350 cc.

Another bit of evidence, also indirect, indicating the quantity of blood perfusing the lungs of the fetal lamb near term, comes from the observations of Barcroft (9) on the amount of blood (z) carried by the ductus arteriosus. The ductus from a 144 day fetus, perfused with cold blood at a pressure of 103 mm. Hg. at the pulmonary and 58 at the aortic end, carried 88 cc. of blood per minute. This quantity may be less than the ductus would carry in situ, but it is certainly of the same order and it is one which is more than a third, probably a great deal nearer a half, of the output of the right ventricle per minute. The output of the fetal lamb heart is probably very near the same as that of the fetal goat, *i.e.*, 0.12 to 0.18 cc. per gram of fetus per minute (19) or about 525 cc. per minute for a 3500 gram fetus or about 262 cc. per minute for the right ventricle. Zeigenspeck (54), one may recall, estimated that at the end of term half of the output of the right heart went through the lungs.

Thus arranged, these data indicate that early in gestation nearly all of the post-caval blood enters the left atrium; a very small part is diverted into the right. At this time very little blood is passing along the aortic isthmus and the ductus arteriosus is carrying very nearly the entire output of the right side of the heart. Under these circumstances there is a wide difference between the oxygen content of the carotid blood and that in the dorsal aorta; the "crossing" of the streams is nearly complete. With advancing development the septum secundum gradually shifts to the right relative to the midpoint of the post-caval channel. As a result of this shift and accompanying it there is a gradual increase in the proportion of the post-caval blood entering the right atrium. Hand in hand with this increase the proportion of the left ventricular output traversing the aortic isthmus increases; the flow through the ductus arteriosus decreases and that through the lungs increases. These changes bring about a gradual increase in the oxygen content of the blood in the right heart and consequently that of the blood traversing the ductus arteriosus and the lung capillaries. As the contribution from the posterior cava via the foramen ovale decreases and the lung circulation opens up, the oxygen content of the blood in the left heart and the carotid gradually fall. Contrariwise the oxygen content of the blood in the dorsal aorta increases as the contribution from the aortic isthmus grows and that from the ductus arteriosus declines. As a result the oxygen content of the carotid blood and that of the dorsal aorta—so different in early development—approach each other as development advances and are equivalent or nearly so at the end of gestation. That this is what does happen is indicated by the studies of the blood gases (13, 17) see figure 3.

The changes in the pattern of the vascular system at birth by caesarian section. The uterine contractions that force the fetus into the external environment also serve to initiate the series of interdependent steps that result in the produc-

of the adult circulation pattern. They serve to impede the placental circulation and to transfer a large part of the placental blood—25 to 30 cc. per kilogram of fetal weight in the case of the lamb (14) and about the same proportion in the human infant (25)—to the fetus. This transfer and the decline in the placental circulation appears to result in the asphyxiation of the fetus and asphyxiation in turn in the initiation of respiration. *In utero* the respiratory center appears to be stimulated by CO_2 excess (52), O_2 want (10) and through the activity of somatic sensory nerves (30, 11, 12). All of these influences must be brought to bear upon the medullary center to an ever increasing degree as the uterine contractions increase in frequency and force. Their relative importance and rôle in initiation of respiration is beyond the scope of this review. Important for the

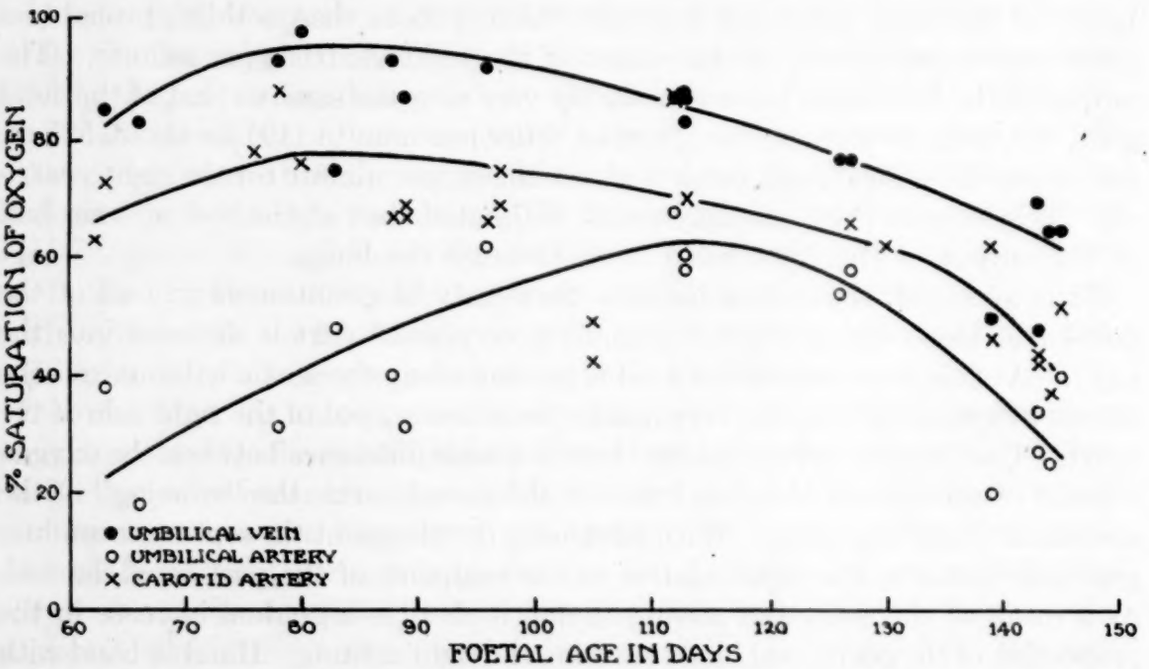

Fig. 3. A graph based upon Barcroft's data illustrating the variations in the oxygen content in the umbilical vessels and in the carotid artery of the fetal lamb as development advances.

present purpose is that they succeed, for the initiation of respiration is a *sine qua non* for the changes in the vascular system. Lamb fetuses whose medullae are destroyed *in utero*, or whose cords are sectioned anywhere between C5 and the calamus scriptorius, always die with the pattern of the circulation unaltered from the fetal type (Barron, unpublished). However, respiratory movements alone do not suffice to produce the change. Lamb fetuses killed *in utero*, after executing respiratory movements, by injecting formalin into the heart always have the fetal pattern of circulation (see Barcroft, 10). The respiratory movements must succeed in ventilating the lungs.

The effects of breathing on the circulation of the fetus have been studied in some detail by Hamilton *et al.* (29) in dogs and rabbits and by Barcroft in sheep. There are minor differences but the main features appear to be shared by all

three fetal types. In dog and rabbit fetuses, before breathing, the systolic pressure is about equal in both ventricles as is the diastolic pressure in the two auricles. During early breathing the diastolic pressure falls in both ventricles and the systolic pressure of the left falls the same distance. The systolic pressure of the right ventricle, however, drops three or four times as far. The pressures rise and fall during the heart cycle but at the lower level. The heart rate is increased. The fall of the diastolic pressure in the two ventricles and the systolic of the left were attributed by Hamilton *et al.* (29) to the decrease in the intrathoracic pressure which results from the expansion of the thorax. The further fall in the right systolic was attributed to a change in the peripheral resistance in the lesser circulation.

In the sheep fetus there is no record of the pressure in the right ventricle but the systemic blood pressure rises 30 or more mm. Hg. (10) with breathing, and the circulation time in the pulmonary circuit falls from 2.7 to 1.4 seconds (8) indicating a decrease in the pulmonary resistance—a decrease that would be compatible with a fall in pressure in the pulmonary artery. The difference in pressure between the pulmonary circuit and the systemic, that is established as a result of breathing, led Hamilton *et al.* (29) to postulate an early closure of the ductus arteriosus. For were it to remain patent, blood would flow along the ductus arteriosus from the aorta to the pulmonary artery which is the opposite direction to that of the fetal stream.

The functional closure of the ductus arteriosus. This postulate has been substantiated for, as indicated by x-ray cine records (4, 5), the ductus arteriosus can and does close in the lamb within four minutes after the placental circulation is arrested and the respiratory passages are opened by the removal of a bag placed on the nose of the fetus before it was taken from the amniotic fluid of the uterus. In these four minutes air had entered the lungs and outlined the bronchial tree in the radiograph. The closure may not always be so abrupt and complete. The ductus may close, reopen and oscillate between complete and nearly complete closure for fifteen or more minutes after ventilation of the lungs is established. The reopening of the ductus appears to occur as the animal deteriorates—as the respiratory movements grow less successful in oxygenating the blood. Simultaneously the circulation time through the lungs increases. In fact there is a suggestion that the degree to which the ductus is closed is directly related to the pulmonary circulation time (8). Both of these happenings are probably correlated with the decrease in the peripheral resistance in the lung and hence with the fall in the systolic pressure in the right ventricle.

This prompt functional closure of the ductus arteriosus associated with the initiation of respiration requires a mechanism that can obliterate the ductus suddenly, and if need be to reopen it; in short, the action of a sphincter. The capacity of the ductus to contract and relax suddenly like a sphincter has been observed directly in full term guinea-pig fetuses (16, 34). With the chest opened, faradic currents applied directly cause it to narrow abruptly as it does if manipulated (16). In the light of these observations and the radiological evidence the view that the ductus is a structure whose functional rôle diminishes

over a relatively long period of time coincident with its anatomical degeneration and obliteration is no longer tenable. The opinion of Gérard (28) that the obliteration of the ductus embraces two stages—1, the physiological occlusion, which occurs very shortly after birth, leaving the ductus patent in all its length but preventing the passage of blood, and 2, the anatomical obliteration—now appears fully justified.

The ductus functions as a sphincter and all recent descriptions of its histology agree that the walls contain structures associated with sphincters (49, 22, 34). According to Von Hayek the tunica media is composed almost entirely of muscle fibers loosely arranged and coiled about the lumen like a long spring. There is an outer layer in which the fibers forming the coil are very nearly circular. The fibers of the inner layer tend to be more longitudinally disposed. These fiber bands are arranged to coil in opposite directions so they cross each other at a near right angle. The elastic tissue layer is very poorly developed and in some regions completely absent.

The studies thus far reviewed appear to answer the questions when and how the ductus closes, but they have raised quite another: How is the closure timed so that the ductus arteriosus narrows and remains so only after the ventilation of the lungs is established and yet may be free to relax again at times during the critical stages of the transition from placenta to lung? There appear to be at least three mechanisms by which the contraction of the musculature of the ductus might be timed and wrought to coincide with the establishment of the ventilation of the lungs, *i.e.*, nervous action, by blood born stimuli, and finally mechanical forces. The first possibility—that of nervous action—was presented and supported with the observations of Barcroft *et al.* (16). They found that stimulation of the peripheral end of the left vagus in guinea-pig fetuses near term produces a blanching of the exposed ductus and a slowing of the heart. Stimulation of the right produced no visible effect on the ductus, though it did slow the heart. Upon these observations the suggestion was advanced that the effect of vagal stimulation is a direct one upon the ductus muscle.

That there are nerve endings in the ductus of the guinea-pig fetus has been established by Kennedy and Clark (34) but they are not numerous nor are they obviously motor in character. Boyd (22) has recently studied intensively the nerve supply to the ductus in a series of fetal forms, including the rabbit, pig, mouse, cat, dog, rat and human. Boyd finds that there are nerve fibers in the ductus musculature, which he suggests may be motor in character, but he makes no statement as to their origin. I have examined serial sections of the ducti of a large number of sheep embryos and fetuses ranging between 26 days and full term. The material had been impregnated by Ranson's pyridine silver method. Known nerve fibers and their endings were satisfactorily impregnated. Sensory endings of the "depressor" type were found in the adventitia near the aortic end but no fibers were found penetrating to the muscle layer or terminating in motor type endings. These studies offer little support for the view that the vagal fibers end on, or directly excite, the ductus musculature.

The question: Can the ductus be functionally closed by direct nervous action,

remains unanswered; but the more important question: Is direct nervous action essential to bring about the closure of the ductus, appears to draw a negative answer from the studies of Kennedy and Clark (35) on the guinea pig. These investigators have apparently severed every known and suspected nerve path between the central nervous system and the ductus. Constriction of the ductus, despite these operations, followed inflation of the lungs with oxygen through a tracheal cannula. As a result of their studies on the denervated ductus, Kennedy and Clark (35) explored the possibilities that its musculature was stimulated by blood-borne substances. In view of their observation that inflation of the lungs with air or oxygen did result in narrowing of the ductus whereas inflation with nitrogen did not, oxygen appeared as a substance, blood borne, that might be effective. To test this possibility bubbles of pure oxygen were introduced into the umbilical vein, a procedure which was promptly followed by the closure of the ductus in four fetuses. Kennedy and Clark (35) suggest the possibility that increased oxygenation of the blood may be the effective stimulus, the oxygen presumably acting directly on the muscle in the ductus. There are, however, certain aspects of these experiments that leave open the possibility that the oxygen was acting not on the ductus but elsewhere, perhaps on the brain.

Oxygen bubbles introduced into the umbilical vein would be taken up by the blood stream, a part of which, if conditions were normal, would reach the ductus only after being diluted by a greater quantity of blood from the superior cava, or the other part only after being forced through the upper circulation having first reached the heart through the foramen ovale. Then, too, there is the objection that the ductus appears to close normally at a time when the oxygen content of the blood is below what it was *in utero* with the placental circulation intact. For example: A lamb after birth by caesarian section may require some hours to get its blood 90 per cent saturated with oxygen (17) yet *in utero*, before the caesarian section was undertaken, its blood may have been saturated to that extent (17). *In utero* the ductus is open; and it certainly is closed in lambs before the oxygen content of their blood returns to the intra-uterine level. Nevertheless, the inherent plausibility of the suggestion of Kennedy and Clark and their evidence supporting it are such that it cannot be set aside by these objections. Their suggestion was put to a further test in the following manner. Arrangements were made to allow warm, whipped sheep's blood saturated with oxygen, to flow into the jugular vein of a full term lamb. The lamb was not removed from the uterus; the neck and vein were exposed through a narrow slit in its wall. The placental circulation remained intact. After 100 cc. of blood had entered the jugular, the most direct route to the ductus, 10 per cent formalin was injected via the same cannula and the fetus killed. Six experiments were carried out. In no one of the six fetuses was the ductus found constricted or even perceptibly narrowed as judged at post mortem (Barron, unpublished). Unless the ductus closed and reopened again when the formalin attacked it, these experiments suggest that an increase in the oxygen content of the blood perfusing the ductus is not sufficient in itself to cause the contraction of the muscle in its walls.

The possibility that substances other than oxygen may stimulate the ductus musculature does not appear to have been extensively investigated. Kennedy and Clark (35) observed that 1 cc. of 1/10,000 adrenalin injected directly into the fetal heart was followed by the closure of the ductus 3 minutes and 1 second after injection started.

There remains to be considered the view that the ductus closes mechanically. There appear to have been some in the past who have regarded the closure of the ductus as due to pressure upon it, or to stretching or other mechanical forces as a result of inflation of the lung and competition for space in the thorax. Such factors may have a rôle but they can no longer be regarded as essential in view of the demonstration of Barcroft *et al.* (16) and Kennedy and Clark (35) that the ductus closes when the chest is open.

But there is yet another mechanical possibility, one outlined by Zeigenspeck (54), *i.e.*, that the musculature of the ductus like that of the umbilical vessels is in a tonic state and that the lumen is kept open by the pressure of the blood in it; that pressure offsets the tendency of the ductus to contract. Any fall in pressure in the ductus according to this view would remove the balance of forces and permit the ductus to narrow. This theory does not appear to have been put to experimental test but there is some evidence to strengthen its probability. Hamilton *et al.* (29) have clearly shown that the systolic pressure in the right ventricle does fall as breathing begins in the rabbit and this fall would appear to be a necessary consequence of breathing in any other fetal type. A fall in the right systolic pressure would reduce the pressure in the ductus. Flow from the aorta, the region of higher pressure after breathing is begun, is prevented in the rabbit and dog by valve-like folds at the aortic end of the ductus. A permanent reduction in pressure is thus established in the ductus which may permit the musculature to contract. Such a mechanism appears to underly the closure of the umbilical veins. Its presence elsewhere would not be surprising.

There is, however, no valve in many forms, for example, the guinea pig (35), sheep (Barron, unpublished) and the human (29) at the aortic end of the ductus which would prevent blood passing from the aorta into the ductus and so maintaining the pressure in the lesser circulation or even raising it, but it has not been established that the pressure does not fall, normally, sufficiently to permit the ductus to narrow. Certainly this possibility is an attractive one for it would offer a simple explanation for the close correlation between the caliber of the ductus and the circulation time through the lung, and would permit an understanding of the failure of the ductus to close in cases of aortic stenosis. Against it there is the observation of Clark and Kennedy (35) that inflation of the lungs with nitrogen is not followed by closure of the ductus in the guinea pig, though when the lungs are inflated with oxygen it does so. The possible effects of nitrogen on the heart and on the systolic pressure in the right ventricle are unknown.

In summary, there is at present no satisfactory explanation of the mechanism initiating the closure of the ductus. One may perhaps be permitted to surmise that the explanation, when it is discovered, will be based on simple hemodynamic considerations.

The functional closure of the foramen ovale. There is general agreement that the functional closure of the foramen ovale is accomplished by the apposition of its valve, the septum primum, to the septum secundum or interatrial septum (see fig. 2). These two structures would appear to be separated when the pressure on the right side of the septum primum exceeds that on its left and approximated when the pressures are equal or reversed. No studies of these pressures prior to the onset of respiration or the impairment of the placental circulation appear to have been made; but so long as a part of the return from the post-caval channel enters the left atrium the pressure in that vessel would appear to be equal to or to exceed the filling pressure via the lung capillaries. A fall, therefore, of the pressure in the post-caval channel or a rise in the filling pressure could serve to close the foramen ovale functionally.

A fall in the pressure in the post-caval channel might be brought about by increased resistance in the capillary bed of the placenta or by constriction of the ductus venosus. Both changes occur at birth. The expansion of the chest and the entrance of air into the lungs may be expected to increase the filling pressure of the left atrium for they reduce the pressure against which the right heart works—from atmospheric to something between atmospheric and intrathoracic—and the lateral pressure on the lung capillaries by replacing solid lung tissue with alveolar air.

Just which one of these changes in pressure or combination of them brings about the initial closure of the foramen ovale has not been determined. At a normal birth the placental circulation is impaired and the fall of the pressure in the posterior cava must precede the ventilation of the lungs. But at delivery by caesarian section the circumstances may be reversed. In the one lamb, delivered and studied radiographically by Barclay *et al.* (7), the foramen ovale was closed within four minutes after breathing began, while the ductus arteriosus was still patent and before the umbilical cord was tied. In this case a rise in pressure on the left side of the septum primum was probably responsible for the closure. This observation makes it clear that the functional closure of the foramen occurs suddenly and not slowly over a long period of time as some investigators have supposed. There is indirect evidence to support this conclusion. Barcroft, Kramer and Millikan (18) observed that the blood in the carotid of a lamb delivered by caesarian section can become completely saturated with oxygen five minutes later if the lamb be given pure oxygen to breathe; a circumstance which preludes any appreciable degree of contamination of the blood in the left atrium.

The closure of the ductus venosus. With the ductus arteriosus and the foramen ovale closed the substitution of the lung for the placenta as a source of oxygen is completed. There remains but the closure of the ductus venosus, the substition of the fetal gut for the placenta as a source of nutrition, before the transition from the fetal to the adult circulatory pattern is completed.

The closure of the ductus venosus does not appear to have attracted the interest that has centered about its counterpart, the ductus arteriosus. Yet its persistence as a patent channel would result in the adult in a condition similar to that attained by Eck's fistula. Anatomical studies (48) (Barron, unpublished)

indicate it is closed by the day following birth. The only records of its functional closure are those mentioned above, the radiographic records of Barclay *et al.* (8). In the case they describe in greatest detail, the ductus was opening and closing ten minutes after delivery by caesarian section; the umbilical cord was undivided and ventilation of the lungs was prevented by a nose bag put on *in utero*. These are not conditions which would appear to permit the closure of either the ductus arteriosus or the foramen ovale; in short they appear to resemble conditions *in utero* more than those of free birth. There may be room for a reasonable doubt that these constrictions in the ductus venosus were a part of the activity associated with the delivery of the fetus from the uterus. Nevertheless there is good reason to suppose that the final closure at birth is wrought by the same mechanism.

In summary, the available evidence indicates that the ductus arteriosus and the ductus venosus are closed functionally within a few minutes after birth by the action of sphincter muscles appropriately placed. It does not permit any conclusion as to how the contraction of these two sphincters is evoked and timed with other aspects of birth. Similarly the evidence indicates that the closure of the foramen ovale may take place abruptly and immediately after birth but the forces responsible for its closure have not been described.

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